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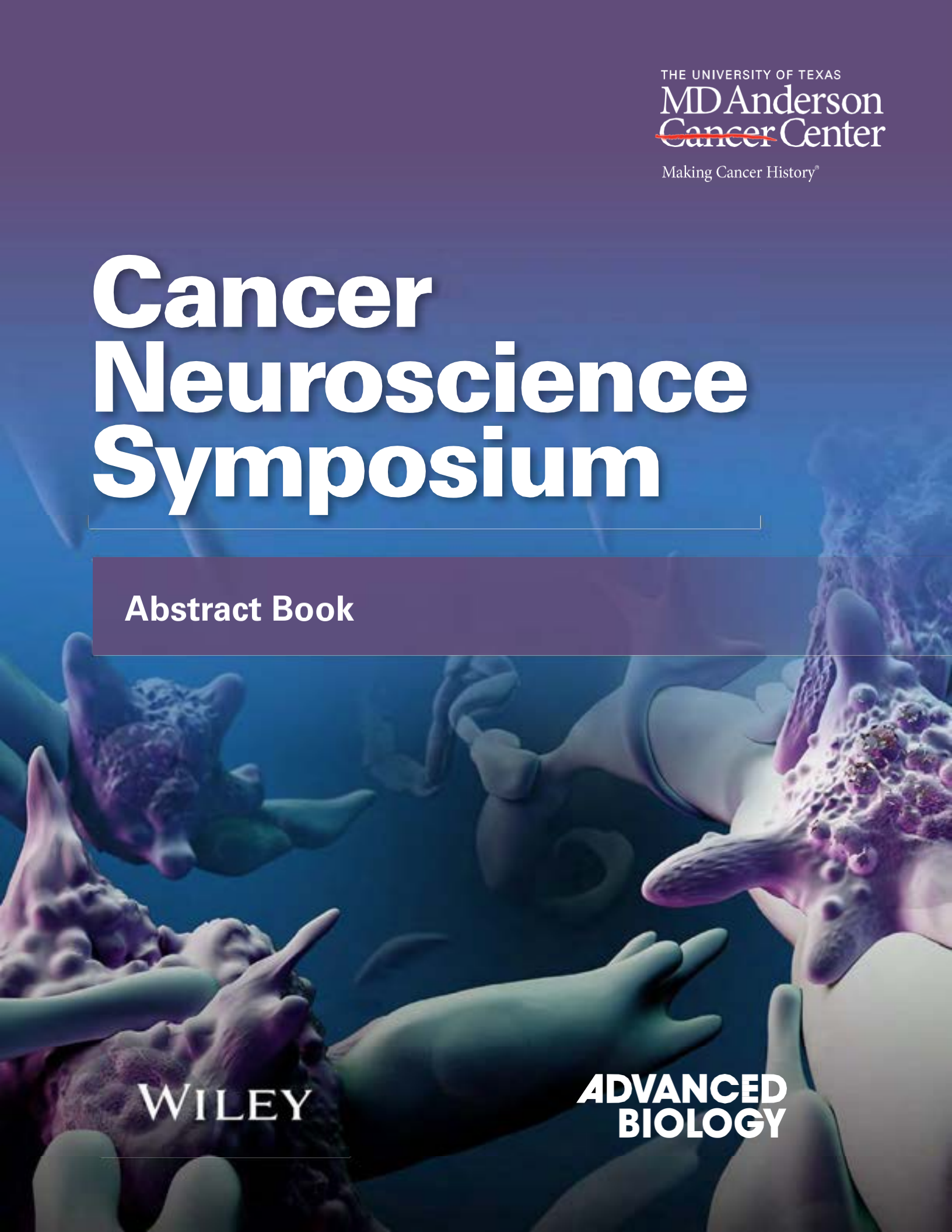
Abstract Book



WILEY

ADVANCED BIOLOGY

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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
University of California at San Francisco, San Francisco, Ca

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

Annabel V Manoleras[1], Aeson Chang[1], Cameron J Nowell[1], Jean Berthelet[2,3], Delphine Merino[2,3,4,5] and Erica K Sloan[1]

Affiliations

1. Monash Institute of Pharmaceutical Sciences (MIPS), Monash University, Parkville, VIC 3052, Australia.
2. Olivia Newton-John Cancer Research Institute (ONJCRI), VIC 3084, Australia.
3. School of Cancer Medicine, La Trobe University, Bundoora, VIC 3086, Australia.
4. Immunology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3052, Australia.
5. Department of Medical Biology, The Faculty of Medicine, Dentistry and Health Science, The University of Melbourne, Parkville, VIC 3010, Australia.

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]

[1]Sunnybrook Research Institute, Toronto, ON; [2]Department of Medical Biophysics, University of Toronto; [3]Department of Laboratory Medicine and Pathobiology, University of Toronto; [4]Princess Margaret Cancer Centre, University Health Network

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 1

A 3D Cortical Microtissue Model for the Study of Glioblastoma Pathology

Dominick Calvao[1], Andrea Schmidt[2], Sean Lawler[2], and Diane Hoffman-Kim[1,3,4]
[1]Institute for Biology, Engineering, and Medicine, Brown University, [2]Department of Pathology and Laboratory Medicine, Brown Cancer Center, Brown University [3]Department of Neuroscience, BrownUniversity, [4]Carney Institute for Brain Science, Brown University

Introduction

Glioblastoma is the most aggressive and the most common form of malignant brain cancer. The median survival from diagnosis is 12-18 months with a five-year survival rate of 5% and 200,000 deaths per year worldwide. Due to its aggressive nature, location, and inter- and intratumoral heterogeneity, glioblastoma is notoriously difficult to both treat and study. There is a strong need for reliable models that recapitulate the inherent heterogeneity and invasiveness of glioblastoma to investigate
the tumor-brain microenvironment and screen therapeutics. Previous research in our labhas demonstrated a 3D cortical spheroid model that exhibits complex intercellular interactions (Dingle, 2015), electrical signaling (Sevetson, 2021), and capillary-like networks (Boutin, 2018) for use in the study of multiple disease states and therapeuticscreening. Here our goal was to establish a 3D *in vitro* model to study glioblastoma pathology.

Methods

Self-assembled spheroids containing 8000 cells/spheroid were derived frompostnatal primary rat cortical tissue. Early-stage (day 2), mid-stage (day 7), and late- stage (day 14) spheroids were seeded with 20,000 GFP-expressing CNS-1 rat glioma cells (Viapiano, 2008) and investigated after 1, 3, and 7 days of co-culture. Spheroids were characterized for neuronal, astrocyte, and microglia response via immunohistochemistry for □-iii-tubulin, glial fibrillary acidic protein, and Iba-1, respectively. Infiltrative behavior of CNS-1 cells was assessed through confocal microscopy.

Results

Our 3D cortical spheroid model exhibited multiple responses to glioblastomacell infiltration. Following co-culture with glioblastoma cells, spheroids contained disrupted □-iii-tubulin(+) neurite networks, as evidenced by punctate staining and blebbing. Spheroids co-cultured with glioblastoma cells also showed evidence of glial reactivity. Astrocytes displayed altered glial fibrillary acidic protein(+) branch morphology, including abnormally organized branch orientation. Iba-1(+) microglia werepresent in higher numbers and with activated morphologies, including the lack of ramified processes present in surveillant microglia. We also examined glioblastomainfiltration in a group of co-culture models at different stages of spheroid maturation.Glioblastoma cells infiltrated more rapidly into early-stage spheroids than late-stagespheroids.

Conclusion

Here we show a novel, high-throughput, 3D cortical-glioblastoma model
that permits the investigation of the interactions between cancer, neural, and glial cells,and the infiltrative behavior of glioblastoma cells.

Significance

There is a significant need for a biomimetic *in vitro* model in the field ofbrain cancer research. Through the use of 3D cortical spheroid - glioblastoma cocultures we can provide a versatile set of models for studying various stages of glioblastoma progression and for testing therapeutics.

Thursday Abstract 2

Spatial transcriptomic characterization of tumor-nerve interactions in pancreatic cancer

Peter L. Wang, Jennifer Su, Carina Shiau, Nicole A. Lester, Ella Perrault, Dennis Gong, DenizOlgun, Ashley Lam, Jimmy A. Guo, Saifur Rahaman, Hannah I. Hoffman, Xunqin Yin, Jaimie L. Barth, Prajan Divakar, Jason W. Reeves, Grissel Cervantes Jaramillo, Carlos Fernandez-del Castillo, Eric Miller, Kathleen Cormier, David D. Ginty, Andrew J. Aguirre, David T. Ting, LeiZheng, Mari Mino-Kenudson, Tyler Jacks, William L. Hwang
Harvard Medical School, Boston, MA.

Introduction

Emerging evidence indicates that the sprouting of new nerve fibers within tumors (tumor innervation) and the infiltration of nerves by malignant cells (perineural invasion or PNI), play important roles in cancer development, metastasis, therapeutic resistance, and death across many cancer types. Pancreatic ductal adenocarcinoma (PDAC), one of the most lethal malignancies with a five-year overall survival of only 11%, has one of the highest rates of nerve involvement (~95% of cases); however, the mechanisms by which cancer cells interact with nervesin PDAC remain incompletely understood.

Methods

To investigate mechanisms of tumor-nerve crosstalk, we used whole-transcriptome digital spatial profiling (DSP) to characterize genes and programs enriched in nerve-proximal versus nerve-distal cancer cells across 435 regions of interest (ROIs) in 29 independent patient- derived PDAC tumors. To examine the potential roles of these genes and programs in tumor-nerve interactions, we utilized CRISPR-engineered mouse PDAC lines overexpressing key transcriptionfactors (TFs) associated with classical (CLS), basal-like (BSL), and the recently identified neural progenitor-like (NRP) malignant cell subtypes and also generated several new lines overexpressingindividual genes identified from our screen. These malignant cell lines were used for co-cultures *in vitro* and for orthotopic transplants *in vivo*. Nerve recruitment, outgrowth, and invasion were assessed with live imaging (Incucyte) and confocal imaging.

Results

Nerve-proximal cancer cells showed an enrichment of 1328 genes, including BSL and NRP malignant subtype programs, and a depletion of 280 genes, including the CLS malignant subtype program, compared to nerve-distal cancer cells. Using CRISPR-engineered lines, we found that conditioned media from tumor cells overexpressing Glis3 (NRP) and Trp63 (BSL) contributed to higher outgrowth of primary sensory neurons *in vitro* compared to conditioned media from cells overexpressing Gata6 (CLS). Glis3-derived tumors also showed increased beta- III tubulin staining *in vivo*. Among malignant cells overexpressing individual gene candidates, Pdgfd-overexpressing cancer cells promoted the highest nerve outgrowth *in vitro* and *in vivo*. Interestingly, targeting Pdgfd-Pdgfrb signaling with a small molecule inhibitor led to reduced invasion of whole explant DRGs by cancer cells.

Conclusion

Using a whole-transcriptome spatial transcriptomic screen, we identified unique tumor-nerve interactions associated with malignant cell subtypes, including a novel Pdgfd-Pdgfrbpathway.

Significance

Future studies aimed at further dissecting and disrupting tumor-nerve crosstalk, including additional unexplored hits and their associated pathways, could result in new therapeuticstrategies to improve outcomes for PDAC patients.

Thursday Abstract 3

Decoding the Intercellular Mitochondrial Transfer at the Nerve-Cancer Interface

Hoover G, Curley O, Obellianne C, Hixson W, Galappaththi S, **Grelet S***

Department of Biochemistry and Molecular Biology, College of Medicine, University of South Alabama, Mobile, AL, USAMitchell Cancer Institute, The University of South Alabama, Mobile, AL, USA.

*Correspondence: **sgrelet@southalabama.edu**

Introduction

Intercellular mitochondrial transfer is a burgeoning topic in cell biology, attracting considerable interest due to its relevance in various pathological conditions, including metabolic disorders, cardiovascular diseases, respiratory diseases, inflammation, neurodegeneration, and cancer. However, the absence of suitable technologies for specifically illuminating cell-cell mitochondrial transfers has left the functional mediators and biological outcomes of these transfers largely unexplored.

Method

To address this knowledge gap, we developed two genetic reporter systems for real-time monitoring of intercellular mitochondrial transfer and tracking the fate of the involved cells *in vitro* and *in vivo*. The MitoREPORTER strategy utilizes a tetracycline-transactivator conditional gene expression system, enabling the real-time tracking of mitochondrial transfer from donor to recipient cells. The MitoTRACER approach employs Cre recombinase to permanently mark recipient cells and their progeny,facilitating lineage tracing of participating cells. These tools open the door to groundbreaking studies on the dynamics of mitochondrial transfer and further investigations into the underlying mechanisms.

Result

To study the nerve-cancer crosstalk established during breast cancer innervation, we developed nerve-cancer cocultures that unveiled the metabolic reprogramming of cancer-associated neurons (CANs), characterized by their increased mitochondrial metabolism. Applying the MitoREPORTER and MitoTRACER approaches to our coculture model, we show how CANs transfer mitochondria to cancer cells, thereby enhancing their oxidative phosphorylation (OXPHOS) capabilities. Utilizing genome-wide screening, we identified key biological mediators of these transfers. Subsequent functional assays, both *in vitro* and *in vivo* highlighted the critical role of the nerve-cancer transfer of mitochondria in the aggressivity of innervated breast cancers.

Conclusion

We have successfully developed a versatile tool enabling high-throughput screening ofcell-cell mitochondrial transfers and their lineage tracing, both *in vitro* and *in vivo*. These tools have shedlight on these transfers as a crucial metabolic support mechanism at the nerve-cancer interface during cancer innervation and have facilitated the identification of its essential mediators.

Thursday Abstract 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* 1,3,4 , Carmen Mota Reyes 1,3,4 , Rouzanna Istvanffy 1,3,4 , Maximilian Kießler 1 , HandeAsimgil 1,2,3,4,, Menar Ekizce 2, Muazzez Gökalp 2, Güralp Onur Ceyhan 2, Ihsan Ekin Demir 1,2,3,4,5

1 Department of Surgery, Klinikum rechts der Isar, Technical University of Munich, School of Medicine,Munich, Germany

2 Department of General Surgery, HPB-Unit, School of Medicine, Acibadem Mehmet Ali AydinlarUniversity, Istanbul, Turkey

3 German Cancer Consortium (DKTK), Partner Site Munich, Germany4

CRC 1321 Modelling and Targeting Pancreatic Cancer

5 Else Kröner Clinician Scientist Professor for Translational Pancreatic Surgery

*Contact information: kaan.cifcibasi@tum.de / 089 4140 6193

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is characterized by pronounced intratumoral nerve growth, neural invasion and neuroinflammation. In our preliminary analysis, we demonstrated mutual trophic effects between myeloid derived suppressor cells (MDSCs) and dorsal root ganglia (DRG) neurons, which suggest a hitherto unconsidered, immunosuppressive role for sensory neurons in PDAC. To elucidate the role of neuron-mediated immune suppression, we characterized the transcriptional profile of DRG neurons innervating tumor-bearing mouse pancreata using spatial transcriptomics.

Methods

DRG isolation and FFPE block preparation were performed from autochthonous (Ptf1a-Cre; LSL-Kras$^{+/G12D}$ [KC], Ptf1a-Cre; LSL-Kras$^{+/G12D}$; Trp53$^{+/fl}$ [KPC], and Ela-TGFα; Ptf1a-Cre; Trp53$^{fl/fl}$; RelA$^{fl/fl}$ [TPAC]) and surgically induced (orthotopic KPC cell implantation and electroporation) mouse models of PDAC. The DRG neurons were subject to spatial transcriptomic profiling at the NanoString GeoMx Digital Spatial Profiling Technology. Immunofluorescence stainings were used to validate the existence of identified mRNA targets on the protein level in DRG neurons.

Results

In our spatial transcriptome analysis, we identified an enriched expression of proteins related to the endopeptidase inhibition pathway in tumor DRGs compared to their age-similar, tumor-free controls. Two of the most enriched ones of these molecules among KPC and TPAC DRGs were *Wfikkn2* (fold change= 1,77 & p< 0,01 for KPC; fold change= 1,73 & p< 0,01 for TPAC) and *Serpinb9b* (fold change= 1,61 & p< 0,01 for KPC; fold change= 1,71 & p<0,01 for TPAC), both previously shown to regulate immune evasion in cancer (*Monestier 2016, Ahmadi 2019, Wang 2021*). Furthermore, we also detected an altered expression of other immunomodulatory pathways, including the chemokine binding. For instance, CXCL15 was highly enriched in both KPC (fold change= 1,45 & p=0,04) and TPAC (fold change= 1,77 & p< 0,01) DRG samples compared to their age-similar, tumor-free controls. Taking the known MDSC recruiting role of this protein into account *(Draghiciu 2015),* its upregulation in tumor DRGs is another strong indication for the immunosuppressive role sensory neurons play in PDAC.

Conclusion and significance

Our spatial transcriptomic analysis of DRGs creates a broad perspective on the diverse roles neurons can play in PDAC and immunomodulation. Further functional and mechanistic studies of targets of interest in PDAC will uncover the true translational potential of targeting sensory neurons for tumor control in PDAC.

Thursday Abstract 5

Sympathetic-sensory nerve coupling in oral squamous cell carcinoma drives tumor progression

Andre Martel Matos, Lisa A. McIlvried, Marci L. Nilsen, Megan A. Atherton, Nicole N. Scheff
Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA

Introduction

Solid tumors are characterized by neo-innervation by peripheral nerve fibers originating from both autonomic and sensory nervous systems. To date, the impact of these two fiber types has been studied independently inthe context of tumor progression and the tumor-associated immune response. We hypothesize that intratumoral sympathetic and sensory nerves interact via neurotransmission to drive immunosuppression andtumor cell proliferation.

Methods

We used a translational approach to test this hypothesis. We prospectively accrued 35 oral cavity head and neck cancer (HNC) patients (69.4 ± 10, 55% male) for the assessment of head and neck cancer patient- reported outcomes, circulating neurotransmitters in the blood, and innervation in HNC tissue. We used a syngeneic orthotopic tongue cancer mouse model in tandem with nociceptive behavior assays, calcium imaging, and immunohistochemistry to understand the underlying mechanism for sympathetic-sensory nervecoupling during tongue tumor progression.

Results

In patients, there was a 2 and 4-fold increase in platelet norepinephrine (NE) from HNC patients with early-andlate-stage tumors compared to healthy donors, respectively. There was a significant positive correlation between NE and patient reported spontaneous ($r=0.634$, $p=0.0001$) but not evoked (i.e. activity-driven) pain. Sympathetic nerves, identified using tyrosine hydroxylase (TH) immunoreactivity, comprised 4.73±1.3% of totalnerve density across tumor samples and there was a positive correlation ($r^2=0.309$) between TH nerve densityand pain. In cancer mice, we found a 4-fold increase in both tongue-innervating ATF3+ (i.e. injured) SCG and TG neurons compared to sham. There was also a 113±38% increase in trigeminal sympathetic innervation paired with a 2-fold decrease SCG *Adra2a* expression, an autoreceptor regulating NE release. There was 15%more excitatory alpha1-adrenergic receptor expression in tongue afferents from tumor-bearing mice and NE evoked a larger (350%) Ca^{2+} transient in more tongue afferents from cancer mice (91%) compared to sham (9%).

Conclusions

In summary these data suggest that tumor burden in the oral cavity can result in a nerve injury response in both sympathetic and sensory neurons resulting in increased sympathetic tone and adrenergic-mediated nociceptive activity resulting in immunosuppression and cancer proliferation.

Thursday Abstract 6

Triple Negative Breast Cancer Hijacks the Sympathetic Nervous System to Resist Chemotherapy

Aeson Chang[1], Edoardo Botteri[2], Steve W. Cole[3,4], Gavin W. Lambert[5,6], Adam K. Walker[1,7,8],Erica K. Sloan[1,4,9]
[1]Drug Discovery Biology Theme, Monash Institute of Pharmaceutical Sciences, MonashUniversity, Victoria, Australia.
[2]Department of Research, Cancer Registry of Norway, Oslo, Norway. [3]Departments of Psychiatry & Biobehavioral Sciences and Medicine, Division ofHematology-Oncology, University of California Los Angeles, CA 90095 USA.
[4]Cousins Center for Psychoneuroimmunology, Semel Institute for Neuroscience and HumanBehavior, and the Jonsson Comprehensive Cancer Center, University of California Los Angeles, CA 90095 USA.
[5]Iverson Health Innovation Research Institute and School of Health Sciences, SwinburneUniversity of Technology, Victoria, Australia.
[6]Human Neurotransmitters Laboratory, Baker Heart & Diabetes Institute, Victoria, Australia.[7]Neuroscience Research Australia, and the School of Psychiatry, University of New South Wales, Randwick, New South Wales, 2031, Australia.
[8]School of Psychiatry, Faculty of Medicine, The University of New South Wales, Sydney,New South Wales, 2052, Australia
[9]Division of Surgery, Peter MacCallum Cancer Centre, Melbourne Victoria 3000, Australia.

Introduction

Beta-blockade has been associated with improved cancer survival in triple negative breast cancer (TNBC) patients. However, the effect of beta-blockade on standard chemotherapy treatment for TNBCremains unclear. To investigate this, we used an integrative approach of pharmacoepidemiology and animal modelling.

Methods

Retrospective pharmacoepidemiologic analyses were conducted to examine the impact of beta-blockeruse on metastatic relapse and cancer-specific survival in TNBC patients. Mouse xenograft models, in vivo bioluminescence imaging, immunohistochemistry, transcriptomic analysis and CRISPR-Cas9 gene editing were used for mechanistic studies.

Results

In TNBC patients who received chemotherapy, multivariate Cox regression modelling revealed beta- blocker use was associated with reduced metastatic relapse, especially in patients receiving anthracycline chemotherapy (adjusted HR=0.49, 95% CI: 0.24-0.99). In mouse xenograft models of TNBC, the beta-blocker propranolol improved the control of metastasis by doxorubicin, an anthracycline, when compared to doxorubicin alone (4T1.2: 60-fold reduction in metastasis burden, $p<0.01$; MDA-231^{HM}: >3-fold reduction, $p<0.05$).
Mechanistic analyses in preclinical models discovered that doxorubicin alone increased sympathetic nerve density (20-fold increase, $p=0.06$, Mann-Whitney test) and increased norepinephrine levels in thetumor (1.3-fold increase, $p<0.05$, Student's t-test). In vitro analyses and gene editing by CRISPR-Cas9confirmed a role of nerve growth factor (NGF) in mediating the neuromodulatory effect of doxorubicin.Sympathetic denervation using 6-hydroxydopamine prior to doxorubicin treatment reduced metastasisburden compared to doxorubicin alone (14-fold reduction, $p<0.05$, repeated one-way ANOVA).
Additionally, transcriptomic and immunohistochemistry analyses on clinical samples revealed that anthracycline chemotherapy amplifies tumour cell response to sympathetic neural signaling by upregulating beta2-adrenoceptor expression. Genetic knockout of beta2-adrenoceptors in tumour cells improved anthracycline control of metastasis in a preclinical model of breast cancer (54-fold reduction, $p<0.01$, repeated one-way ANOVA).

Conclusion

These findings reveal an unanticipated neuromodulatory effect of anthracyclines that may undermine their therapeutic efficacy. Supplementing anthracyclines with adjunctive beta2-adrenoceptor antagonists represents a novel therapeutic strategy to improve treatment of TNBC.

Significance to cancer neuroscience field

Previous published work by others has shown cancer cells can reprogram neurons in the tumor (Amit, 2020) and drive neurogenesis in tumors by recruiting neural progenitors from the central nervous system(Mauffrey, 2019). The current work extends these findings by showing that cancer-neuron interactionscan be impacted by standard cancer treatments. We found that in response to chemotherapy, breast cancer cells promote sympathetic innervation in tumors and are sensitized in their response to sympathetic neural signaling.

Thursday Abstract 7

Early-stage pancreatic cancer associated exosomes contribute toparaneoplastic neurological syndrome.

Krystal English[1], Ph.D., Kathleen McAndrews[1], Ph.D., Amanda Haltom[1], Ph.D., Jiha Kim[1], Ph.D., Patience Kelly[1], Barbara Moreno Diaz[1], Janine Hensel[1], Ph.D., Carolina Ruivo[1], Ph.D., Jennifer Lopez[1]., Raghu Kalluri[1,2,3], M.D., Ph.D.

1 Department of Cancer Biology, The University of Texas MD Anderson Cancer Center, Houston, TX
2 Department of Bioengineering, Rice University, Houston, TX
3 Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX

Introduction

Exosomes are membrane-bound nanovesicles known to be secreted by various cells, including pancreatic ductal adenocarcinoma cells (PDAC). In the context of pancreatic cancer, exosomes are thought to play important roles in cellular communication during metastasis and paraneoplastic disorders. Paraneoplastic disorders are clinical symptoms based on multiple causes associated with occult malignancy without metastatic dissemination. Paraneoplastic neurologicaldisorders most associated with pancreatic cancer include neuropathic pain, prodromal depression, neuroinflammation-associated cachexia, and cognitive dysfunction. Investigations into the role of exosomes in paraneoplastic neurological disorder have yet to be done. Using genetically engineered mouse models we have observed the phenomenon of pancreatic cancer derived exosomes coalescingamongst neurons. From these findings, we hypothesize that pancreatic cancer exosomes from PDAClesions contribute to paraneoplastic neurological syndrome.

Methods

To investigate this hypothesis, we have used a genetically engineered mouse model of spontaneous PDAC where only PDAC cells express cytoplasmic YFP, and exosomes (which express CD9) derived from these cells are tagged with mCherry. Based on preliminary data we quantified the percentage of neuronal cells with exosome coalescence with mCherry-CD9 signaling between normal and pancreatic. Using TUNEL assay we quantified the percentage of neurons undergoing cell death, and then used immunofluorescence to observe exosome presence amongst neurons undergoing cell death.

Results

Interestingly, we saw that Purkinje and hippocampal neurons had an increased density of mCherry-CD9 tagged exosomes thus were selected for quantification. We saw a significant difference between the mean of normal vs. pancreatic cancer ($p=0.0034$). We also observed significant cell deathvia TUNEL assay of normal vs. pancreatic cancer ($P<0.0001$) including the presence of “empty baskets”. Further immunofluorescence indicated caspase 7 positivity amongst the Purkinje cell layer indicating potential death of basket cells and Bergmann glia.

Conclusions

The current results demonstrate that pancreas-derived exosomes not only enter the brain, coalesce around hippocampal and Purkinje neurons, but may be involved in neuronal cell death. Future work is focused on elucidating the microglial morphological changes between normal and pancreatic cancer. Moreover, we are performing behavioral experiments to explore the effects of pancreatic cancer exosomes on movement, which may provide evidence of exosome induced paraneoplastic effect.

Significance to cancer neuroscience

Paraneoplastic neurological disorders are rare but devastating side effects of cancer, including pancreatic cancer. Despite strong documentation there has been littledone to elucidate potential causes associated with the development of paraneoplastic neurological disorders. This study will provide a groundbreaking contribution to understanding the role of exosomes in propagating paraneoplastic neurological disorders that afflict cancer patients.

Thursday Abstract 8

Dissecting tumor-promoting crosstalk between enteric glia and colon cancer.

Katherine Letai[1], Marissa Scavuzzo[1], Aritra Nag[1], Bruce Armstrong[1], Peter Scacheri[1,2], PaulTesar[1]
[1]Department of Genetics and Genome Sciences, Case Western Reserve University Schoolof Medicine, Cleveland, OH, USA, [2]Amgen Inc., Thousand Oaks, CA, USA
Presenting Author: Katherine Letai, kcl68@case.edu

Introduction

Tumor cells can trigger a transcriptional and functional state change in neighboring cells, promoting the transition of these cells into cancer-associated, reactive cells. In the brain, glia can be reprogrammed by cancer cells to secrete factors that enhance the tumor's invasive capacity. Glia are also present in the intestine, as the predominant cell type of the enteric nervous system, but their interactions with colorectal tumors are not well-defined.

Enteric glia usually promote healthy gut processes such as epithelial homeostasis and immune response. However, they are transcriptionally plastic and acutely sensitive to their environment. Previous work suggests enteric glia can support tumor growth both *in vivo* (Yuan, 2020) and *in vitro* (Valès, 2019) but the underlying mechanisms remain unclear. In this project, we hypothesize that enteric glia become reactive in the presence of cancer andcontribute to tumor growth.

Methods

To define glia-tumor crosstalk in colorectal cancer, we have coupled *in vivo* snRNA-seq with primary *in vitro* functional manipulation. Tumor and normal colon from *PIK3CA$^{H1047R/+}$Kras$^{G12D/+}$Apc$^{f/+}$Cdx2p-CreERT2* and wildtype mice were sequenced to examine glial populations in the cancer microenvironment. We also developed a novel coculture system combining primary mouse enteric glia and colon tumoroids, providing a refined platform to parse glia-cancer dynamics.

Results

RNA sequencing of single nuclei reveals shifts in the heterogeneous enteric glial population when a colon tumor is present. Interactome analysis also predicts paracrine interactions between these enteric glia and other cells in the tumor microenvironment. In the coculture system, both glia and tumoroids undergo phenotypic changes: glia show elevated levels of known reactive markers when exposed to tumoroids, while the tumoroids grow more rapidly.Bulk RNA-seq of the tumoroids reveals multiple candidate pathways driving this response to enteric glial signals.

Conclusion

This research highlights the pathogenic bidirectional interactions between glia and colon tumors. We are poised to use the coculture system to probe functional importance of the enteric glial changes identified by snRNA-seq, potentially revealing new therapeutic targets.

Significance to the cancer neuroscience field

The most extensive study of communication between enteric glia and colon cancer to date, this work highlights the importance of cancer neuroscience beyond the central nervous system and beyond neurons. Understanding glia-tumor interactions in colon cancer may create an opportunity for cancer neuroscience principles to ameliorate the burden of the 2ndleading cause of cancer death in the US.

Thursday Abstract 9

Elucidating the role of sympathetic nerve crosstalk with cancer associated fibroblasts in PDAC

Ariana Satler, Parham Diba, Tetiana Korzun, Mara Sherman, Teresa Zimmers & Ece Eksi
Oregon Health & Science University, Portland, OR.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy fueled by its tumor microenvironment. Within the complex tumor microenvironment, nerves are emerging as facilitators of tumorigenesis, progression, and metastasis; however, the mechanisms and mediators of the distinct peripheral nerves remains understudied. An increasing amount of evidence indicates that the dynamic interactions of sympathetic nerves with different cell types in the tumor microenvironment could contribute to tumor progression.

Results

In congruence with other studies, we found that cancer associated fibroblasts (CAFs) upregulate a variety of neuro-modulating genes, however the impact of sympatheticnerves on CAFs and how this contributes to tumor-promoting phenotypes is unclear. Our preliminary imaging of murine and human PDAC tumors show that intratumor sympathetic nerves are usually surrounded by an abundance of CAFs. Furthermore, our *in vitro* cocultures of murine CAFs and primarysympathetic nerves show that CAFs consistently reach out and interact with the nerve fibers. Now we have embarked on several studies in which we evaluate the transcriptional and phenotypic shifts resulting from the interactions between sympathetic nerves and CAFs *in vitro* and *in vivo.* We hypothesize that the crosstalk between sympathetic nerves and CAFs, enhances tumor promoting phenotypes in PDAC.

Conclusion

Overall, with these studies, we aim to contextualize the bi-directional crosstalk between sympathetic nerves and CAFs, and the resulting contributions to PDAC initiation and progression.

Thursday Abstract 10

Identifying central mechanisms of glucocorticoid circadian rhythm dysfunction in breast cancer

Adrian M. Gomez, Yue Wu, Chao Zhang, Leah Boyd, Adrian Berisha, Lucas M. Cheadle,Jeremy C. Borniger
Cold Spring Harbor University, Cold Spring Harbor, NY

Introduction

The circadian release of endogenous glucocorticoids (GCs) is essential in preparing and synchronizing the body's daily physiological needs. Disruption in the rhythmic activity of GCs has been observed in individuals with a variety of cancer types, and blunting of this rhythm predicts cancer mortality and quality of life. This suggests that a disrupted GC rhythm is potentially a shared phenotype across cancers. However, whether this disrupted rhythm is conserved in rodent models, and the causal mechanisms that link GC rhythm dysfunction and cancer outcomes remain preliminary at best. The regulation of daily GC activity has been well-characterized and ismaintained, in part, by the coordinated actions of the hypothalamic-pituitary-adrenal (HPA) axis, consisting of the suprachiasmatic nucleus (SCN) and corticotropin-releasing hormone-expressingneurons of the paraventricular nucleus of the hypothalamus (PVN^{CRH}). Consequently, we set out to examine if cancer-induced GC dysfunction is regulated by disruptions within these hypothalamic nuclei.

Results

In comparison to their tumor-free baseline, mammary tumor-bearing mice exhibited a blunting of GC rhythms across multiple timepoints (with the most prevalent divergence occurring at the rhythmic peak) throughout the day, as measured by the overall levels and the slope of fecal corticosterone rhythms during tumor progression. Additionally, DREADD-mediated stimulation of PVN^{CRH} neurons at the peak of normal GC activity decreased tumor size and weight,whereas stimulation of PVN^{CRH} neurons at the trough of normal GC activity failed to do so; suggesting that normalization of glucocorticoid rhythms may be important in tumor outcomes. We then examined how peripheral tumors shape HPA activity. *In vivo* fiber photometry data show that the activity of PVN^{CRH} neurons exhibit enhanced calcium activity during tumor progression, as compared to baseline (no tumor) activity, while calcium activity within the SCN failed to show a difference between tumor-bearing mice and mice without tumors. Further, we also found no discernable differences in pituitary or adrenal function, indicating that tumors may be specificallytargeting the PVN^{CRH} neurons of the HPA axis to exert glucocorticoid rhythm disruption. Current studies are utilizing slice electrophysiology and bulk RNA sequencing to determine how tumors may be altering the activity of PVN^{CRH} neurons.

Conclusions

Taken together, this suggests that there may be a faulty HPA response during tumor progression, particularly within the PVN^{CRH} neurons. Understanding how tumors disrupt GC rhythms and the HPA axis may better provide a mechanistic understanding for the systemic issues faced by cancer patients.

Thursday Abstract 11

B Cell Phenotype Is Altered By Neuronal Signaling In ColorectalCancer

Meike S. Thijssen[1,2], Simone L. Schonkeren[1], Musa Idris[1,3], Joëlle de Vaan[1], Linde Coolkens[1], Jaleesa van der Meer[1], Kim Wouters[1], Lieve Temmerman[4], Pieter Goossens[4], Frank Verhaegen[5], Ludwig Dubois[6], Carmen López-Iglesias[7], Marion J. Gijbels[1,8], WerendBoesmans[1,2], Veerle Melotte[1,3]

1. Department of Pathology, GROW – School for Oncology and Reproduction, Maastricht University MedicalCenter, Maastricht, The Netherlands

2. Biomedical Research Institute (BIOMED), Hasselt University, Hasselt, Belgium

3. Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands

4. Department of Pathology, CARIM – School for Cardiovascular Diseases, Maastricht University Medical Center,Maastricht, The Netherlands

5. Department of Radiation Oncology (Maastro), GROW – School for Oncology and Reproduction, MaastrichtUniversity, Maastricht, The Netherlands

6. The M-lab, Department of Precision Medicine, GROW – School for Oncology and Reproduction, MaastrichtUniversity, Maastricht, The Netherlands

7. Division of Nanoscopy, Maastricht MultiModal Molecular Imaging Institute, Maastricht University, Maastricht, The Netherlands

8. Department of Medical Biochemistry, Experimental Vascular Biology, Amsterdam UMC, Amsterdam, TheNetherlands

Introduction

The importance of neuron-cancer crosstalk in colorectal cancer (CRC) has become widely recognized over the last years (Vaes, 2022). Enteric neurons can impact CRCvia the release of extracellular matrix proteins, however, the influence of enteric neurons on the cellular and molecular profile of CRC remains unknown.

Methods

To study the effect of enteric neuron density on CRC, hypo-innervated ($Hand2^{fl/+}$;Wnt1-Cre2) and control ($Hand2^{fl/+}$) mice were subjected to a colitis-associated CRC induction protocol using azoxymethane and dextran sodium sulfate. After isolated tumor tissues were subjected to RNA sequencing, followed by a gene set enrichment analysis for thehallmarks gene set and for hallmarks of cancer genes. After, stool samples were analyzed formicrobiota composition using 16S rRNA sequencing and flow cytometry was performed to assess immune cell populations. Immunolabeling and transmission electron microscopy were used to localize B cells and tumor cells, which interaction was further investigated in vitro.

Results

Neuronal density did not affect tumor number, size or burden. RNA sequencing of isolated tumors depicted a clear difference in tumor transcriptome between hypo-innervated and control mice, with a high number of immunoglobulin-related differentially expressed genes and an enrichment of the gene ontology (defense) response to bacteria. Moreover, gene set enrichment analysis indicated that the cancer hallmark ‘avoiding immune destruction’ was enriched for differentially expressed genes. 16S rRNA sequencing of stool did not reveal differences in the intestinal microbiome. However, flow cytometry experiments depicted a significant reduction of B cells (CD45+B220+) in the hypo-innervated versus the wildtype mice. More specifically, germinal center B cells (CD45+B220+CD19+GL7+) were significantly reduced, whereas immunoglobulin D expression on B cells was upregulated. Double immunolabeling showed that B cells localize mostly in the colonic submucosa, where they appear in close proximity to nerves. This was confirmed by transmission electron microscopy,and suggests direct communication. Preliminary data from flow cytometric analysis of B cells stimulated with the nicotinic agonist dimethylphenylpiperazinium (DMPP) in vitro showed increased proliferation and altered B cell subtype abundance compared to unstimulated B cells, suggesting that acetylcholine signaling is involved in the B cell effects observed in our in vivo model. Currently, we are investigating the effect of these nerve-stimulated B cells on CRC in vitro.

Conclusion and Significance

This is the first study investigating the effect of neuronal densityon the cellular and molecular profile of CRC and presents a potential interaction between neurons and B cells in the context of CRC.

Thursday Abstract 12

The Role of the Neuroimmune Axis in Hepatocellular Carcinoma: Insights from a Rodent Model

Santosh K. Mandal, Rahul A. Sheth
The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction

There is a growing appreciation for the influence of the peripheral nervous system on cancer progression (Globig 2023, Mancusi 2023). Modulation of sympathetic signaling to the liver may represent a new therapeutic approach for hepatocellular carcinoma (HCC). The purpose of this study was to investigate ina preclinical model the effects of sympathetic neurolysis combined with systemic checkpoint inhibition in HCC tumors.

Methods

An HCC model was established using orthotopic subcapsular implantation of 1 x 10^7 HCA-1 hepatoma cells into the livers of C3H mice through mini-laparotomy. After achieving the target tumor volume, mice were randomized into neurolysis and sham groups. A neurotoxin, 6-hydroxydopamine (6-OHDA), was administered intraperitoneally at a dose of 200 mg/kg for five consecutive days to ablate peripheral sympathetic nerve terminals. Mice were further randomized to receive either anti-PD1 antibody or vehicle control. The PD-1 inhibitor was administered intraperitoneally at 250 µg per injection for five consecutive days. On day 7, the animals were euthanized, and samples of blood, liver, and spleen tissues were collected for analysis. Liver tumor sections were processed for conventional H&E staining and spatial phenotyping using Lunaphore/Opal staining. Flow cytometry was employed to analyze various immune cell types in the liver tumor and blood samples.

Results

Intraperitoneal injection of 6-OHDA successfully ablated sympathetic nerve fibers in both the liver and liver tumors. This was confirmed via anti-tyrosine hydroxylase, NF-H, and CD31 staining. Neurolysis altered the tumor immune microenvironment by reducing inhibitory signals generated by myeloid- derived suppressor cells. This was further corroborated by increased infiltration of activated effector CD8+ T cells, helper CD4 cells into the primary tumor site in the combination 6-OHDA+PD-1 inhibitor arm. The combination treatment also led to an increased ratio of CD8+T cells to Treg cells and decreased colocalization of CD8+ T cells with immunosuppressive cells in the tumor microenvironment.

Conclusion

Neurolysis effectively modulates the local tumor immune microenvironment, thereby enhancing theefficacy of immune checkpoint inhibition in HCC tumors.

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Thursday Abstract 13

Glioblastoma disrupts cortical network activity at multiple spatial and temporal scales

Jochen Meyer[1,3, 6], Kwanha Yu[2,5,6], Estefania Luna-Figueroa[2], Ben Deneen[2,3,5,6], Jeffrey Noebels[1,3,4,6]*
[1]Department of Neurology; [2]Center for Cell and Gene Therapy; [3]Department of Neuroscience; [4]Department of Human and Molecular Genetics; [5]Department of Neurosurgery, [6]Center for CancerNeuroscience, Baylor College of Medicine, Houston, TX, USA

Introduction

The emergence of glioblastoma (GBM), the most aggressive form of brain cancer, initiates early and persistent neural hyperexcitability with signs ranging from mild cognitive impairment to convulsive seizures. GBM exploits the complex interplay between neurons and glia to create a microenvironment favorable for its own expansion yet hostile to network excitability homeostasis, impeding efforts to achieve long-term clinical remission. At the leading edge, a complex sequence of molecular, cellular and synaptic remodeling in the tumor microenvironment (TME) with early loss and impairment of interneurons promotes epileptogenesis and impairs cognitive processes. Distinguishing between healthyand epileptic tissue during tumor resection, as well as defining whether network hyperexcitability in areas more distant than 1 mm from the tumor margin is recruited and how this remodeling can be suppressed to regain healthy cortical function remain major treatment challenges.

Methods

We combined cellular and widefield imaging of calcium and glutamate fluorescent reporters in two GBMmouse models with distinct synaptic microenvironments and infiltration profiles over a prolonged 3- month period of tumor expansion in awake mice. Simultaneously recorded EEG, locomotion, whisker and eye movements allowed us to control for the behavioral and attentional state of the animals during imaging. We analyzed peritumoral activity both mesoscopically at low spatial magnification/high temporal resolution covering both hemispheres, and at high magnification (2-photon excitation microscopy) in the same animals to pinpoint the distance of individual neurons from tumor cells at the leading edge.

Results

Functional metrics of neural ensembles are dysregulated during tumor invasion depending on the stageof malignant progression and tumor cell proximity. Neural activity is differentially modulated during periods of accelerated and inhibited tumor expansion. Abnormal glutamate accumulation precedes andoutpaces the spatial extent of baseline neuronal calcium signaling, indicating these processes are spatially and temporally uncoupled in tumor cortex.

Conclusions

Our data reveal a nonlinear sequence of changes in functional network connectivity over time, a clear genetic dissociation between the speed of tumor invasion, and a strong correlation between accelerated local cortical tumor expansion and neuronal activity gradients along distance from the tumor margin. These results indicate an unexplained but important difference in the way the specific genetic makeupof GBM may influence how it interacts with the surrounding microenvironment.

Significance to the cancer neuroscience field

Our current understanding that tumor genetics drive progression of the cortical hyperexcitability microenvironment and hence impairment of higher order cortical function predicts that matchingprecision GBM diagnoses with information on residual disease can help guide future tailored management of the neurological comorbidity of brain tumors.

Thursday Abstract 14

NKCC1-dependent GABAergic signaling drives neuronal hyperexcitability and tumor progression in glioblastoma

Saritha Krishna, Cesar Nava Gonzales, Andy Daniel, Clara Siebert, Jasleen Kaur, Mia Salans, Vardhaan Ambati, Abrar Choudhury, Thy Vu, Arpana Mckinney, Pavithra Viswanath, Jeanette Hyer, Shawn Hervey-Jumper
University of California, San Francisco, San Francisco, CA, USA

Introduction

A hallmark of TAE is the hyperexcitability of neurons adjacent to the tumor; however, the mechanisms underlying the hyperactivity of neurons within the tumor (glioma- intrinsic neurons) remain poorly characterized. Single-cell RNA sequencing (13,730 cells analyzed) in this system identified several circuit assembly and GABA signaling genes elevated in glioma-intrinsic neurons, including the sodium-potassium-chloride co-transporter 1 (NKCC1), which mediates chloride (Cl^-) influx into cells. This elevated NKCC1 expression and increased intracellular Cl^- levels create a less negative potential for GABA, promoting a depolarizing and functionally excitatory GABAergic response. Here we test the hypothesis that the hyperexcitable behavior of glioma-intrinsic neurons in GBM is mediated by elevated NKCC1, resulting in increased GABAergic transmission, and that inhibiting NKCC1 rescues the neuronal hyperresponsive signature and aggressive tumor phenotype.

Methods

Single-nucleus sequencing (sNuc-Seq) was performed on neuron-glioblastoma co-cultures (54,000 cells analyzed) to determine whether genes involved in GABAergic synaptic transmission are involved in glioma-mediated neuronal responses. The network dynamics and synchronous activity of neurons co-cultured with NKCC1-high and low-expressing GBM cells were captured using live- cell calcium imaging and multielectrode array (MEA). Mechanistic and functional studies using calcium and chloride imaging for validating therapeutic vulnerabilities to NKCC1 silencing by shRNA knockdown and by the FDA-approved drug bumetanide were performed *in vitro*. Patient-derived xenograft (PDX) *in vivo* experiments evaluated the effect of NKCC1 on tumor growth andprogression.

Results

sNuc-seq revealed an upregulation of neuronal NKCC1 when co-cultured with NKCC1-high-expressing GBM cells. Interestingly, this increased neuronal NKCC1 expression correlated with increased neuronal $GABA_A$ receptor expression, indicative of the strong association between neuronal NKCC1 and GABAergic signaling. NKCC1-high primary patient-derived GBM cells showed increased Ki67 proliferation index in both *in vitro* neuron- glioma co-culture model and *in vivo* PDX model. Electrophysiological analysis of glioblastoma- neuron co-cultures using MEA and calcium imaging demonstrated increased network burst synchrony in the presence of NKCC1-high expressing tumor cells. This increase was eliminated in the presence of NKCC1 inhibition using bumetanide and shRNA knockdown. The reduction in neuronal synchrony induced by NKCC1 downregulation correlated with a significant decrease inintracellular Cl^-.

Conclusion

These findings reveal a novel mechanism driving neuronal hyperactivity in glioblastoma and also identify potential therapeutic vulnerabilities.

Significance

We expect that the results from this work will have an important positive impact as they build on current theories of neural regulation of cancer related to cortical hyperexcitability.

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Thursday Abstract 15

Glioma-Induced Alterations in Excitatory Neurons are Reversed by mTOR Inhibition

Alexander R. Goldberg*, Athanassios Dovas*, Daniela Torres*, Sohani Das Sharma, Angeliki Mela, Edward M. Merricks, Markel Olabarria, Leila Abrishami Shokoohm, Hanzhi T. Zhao, Corina Kotidis, Peter Calvaresi, Ashwin Viswanathan, Matei A. Banu, Aida Razavilar, Tejaswi
D. Sudhakar, Ankita Saxena, Cole Chokran, Nelson Humala, Aayushi Mahajan, Weihao Xu,Jordan B. Metz, Cady Chen, Eric A. Bushong, Daniela Boassa, Mark H. Ellisman, Elizabeth
M.C. Hillman, Guy M. McKhann II, Brian J. A. Gill, Steven S. Rosenfeld, Catherine A.Schevon, Jeffrey N. Bruce, Peter A. Sims, Darcy S. Peterka, Peter Canoll.
*These authors contributed equally
Columbia University, New York City, NY

Introduction

Gliomas are highly aggressive brain tumors characterized by poor prognosis and composed of diffusely infiltrating tumor cells that intermingle with non-neoplastic cells in the tumor microenvironment, including neurons. Neurons are increasingly appreciated as important reactive components of the glioma microenvironment, due to their role in causing hallmark glioma symptoms, such as cognitive deficits and seizures, as well as their potential ability to drive glioma progression. Separately, mTOR signaling has been shown to have pleiotropic effects in the brain tumor microenvironment, including regulation of neuronal hyperexcitability. However, the local cellular-level effects of mTOR inhibition on glioma-induced neuronal alterations are not well understood.

Methods and Results

Here we employed neuron-specific profiling of ribosome- bound mRNA via ‘RiboTag,’ morphometric analysis of dendritic spines, and in vivo calcium imaging, along with pharmacological mTOR inhibition to investigate the impact of glioma burden and mTOR inhibition on these neuronal alterations. The RiboTag analysis of tumor-associated excitatory neurons showed a downregulation of transcripts encoding excitatory and inhibitory postsynaptic proteins and dendritic spine development, and an upregulation of transcripts encoding cytoskeletal proteins involved in dendritic spine turnover. Light and electronmicroscopy of tumor-associated excitatory neurons demonstrated marked decreases in dendritic spine density. In vivo two-photon calcium imaging in tumor-associated excitatory neurons revealed progressive alterations in neuronal activity, both at the population and single-neuron level, throughout tumor growth. This in vivo calcium imaging also revealed altered stimulus- evoked somatic calcium events, with changes in event rate, size, and temporal alignment to stimulus, which was most pronounced in neurons with high-tumor burden. A single acute dose ofAZD8055, a combined mTORC1/2 inhibitor, reversed the glioma-induced alterations on the excitatory neurons, including the alterations in ribosome-bound transcripts, dendritic spine density, and stimulus evoked responses seen by calcium imaging. These results point to mTOR- driven pathological plasticity in neurons at the infiltrative margin of glioma – manifested by alterations in ribosome-bound mRNA, dendritic spine density, and stimulus-evoked neuronal activity.

Conclusions

Collectively, our work identifies the pathological changes that tumor-associated excitatory neurons experience as both hyperlocal and reversible under the influence of mTOR inhibition, providing a foundation for developing therapies targeting neuronal signaling in glioma.

Thursday Abstract 16

Sensory Stimulation Induces Microglial Activation and Glioma Cell Proliferation through Purinergic Signaling

Alexander R. Goldberg*, Corina Kotidis*, Cady Chen*, Michael Lahiff, Brianna Pereira, Nkechime E. Ifediora, Athanassios Dovas, Angeliki Mela, Nelson Humala, Aayushi Mahajan,Jaeseung Lee, Alex Eaker, Brian J.A. Gill, Catherine Schevon, Osama Al-Dalahmah, Darcy S.Peterka, Peter Canoll
*These authors contributed equally
Columbia University, New York City, NY.

Introduction

Glioma patients typically present with neurological symptoms, such as seizures and cognitive deficits, and recent studies have implicated neuronal activity as a potential driver of glioma progression. While stimulation of tumor-associated excitatory neurons via optogenetics or chemogenetics has been shown to increase glioma cell proliferation, the consequences of stimulating neuronal activity via physiologic sensory stimulation is incompletely understood. Furthermore, gliomas grow in complex microenvironments in which tumor cells intermingle with non-neoplastic cells, such as neurons and microglia, and the extent to which neuronal activity impacts glioma cells directly versus indirectly through other cell types is not known. Extensive bidirectional communication occurs between neurons and microglia and microglia are often the most abundant cell at the invasive glioma margin.

Results

Neuronal activity modulates microglial motility, activation, and morphology via purinergic signaling in which neurons secreteATP in an activity-dependent manner and microglia bind this ATP at purinergic receptors. Immunohistochemical analysis revealed that P2RX7 levels are significantly elevated in both tumor margin and core in our mouse glioma model. We hypothesized that increased physiologic-level neuronal activity, via whisker stimulation, would modulate both glioma cells and tumor- associated microglia. Analysis revealed significant increases in Iba1+ area following a seven- hour whisker stimulation protocol, which was significantly reduced by a single dose of PPADS, a broad purinergic inhibitor, given prior to stimulation. Furthermore, whisker stimulation causeda significant increase in glioma cell proliferation, measured by EdU labeling index. PPADS administration inhibited this whisker stimulation induced increase in glioma cell proliferation at the outermost margins of the tumor. Next, we performed both ex vivo and in vivo imaging of intracellular calcium dynamics of tumor-associated microglia. Ex vivo imaging found that tumor-associated microglia respond to ATP in a concentration-dependent manner and that PPADS significantly reduces these responses. Similarly, in vivo microglial calcium imaging during whisker stimulation revealed that tumor-bearing mice displayed increased microglial calcium transients in and around the glioma-infiltrated cortex and reduced activity farther from the tumor margin. Ongoing morphometric, transcriptional, and in-situ hybridization experimentsaim to further understand this neuron-microglia-glioma relationship.

Conclusions

Together, our work demonstrates that glioma-induced alterations in neuronal activity induce changes in microglia and glioma cells at the tumor margin. Furthermore, these effects can be ameliorated through targeting purinergic signaling pathways, pointing toward new strategies to treat and slow tumor progression.