3089

Clinicopathologic and Prognostic Significance of LGR5, a Cancer Stem cell Marker in Peritoneal Metastasis of a Colorectal Origin

Chukwuemeka Ihemelandu
Georgetown - Howard Universities

OBJECTIVES/SPECIFIC AIMS: Leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5) is expressed on Wnt/β-catenin-dependent adult stem cell populations of the colon. Cancer stem cells are hypothesized to be the driving force behind tumor progression and metastasis, making them attractive therapeutic targets. Our aim was to analyze the clinicopathologic and prognostic significance of LGR5 expression in a cohort of colorectal cancer patients with peritoneal metastasis. METHODS/STUDY POPULATION: A total of 49 Formalin-fixed paraffin-embedded (FFPE) tissue blocks of primary or metastatic tumors and their respective normal tissues were collected from the tissue bank for time period 2009-2015. LGR5 expression was assessed at the protein level through immunohistochemical (IHC) staining of tissue microarray (TMA) constructs consisting of pairs of tumor and normal colon tissue. The correlation between LGR5 expression and clinicopathologic parameters and prognosis was assessed by statistical analysis. RESULTS/ANTICIPATED RESULTS: Of the 49 patient sample, 30(61.22%) were female vs. 19 (38.78%) males. Age range at initial diagnosis ranged from 31.7 years to 84.4 years, with a median age of 61.29 years. Duration of follow-up ranged from 1 – 9 years with a median of 5 years.LGR5 expression was higher in colorectal cancer than in normal mucosa. In univariate survival analysis overexpression of LGR5 was significantly associated with improved survival (p=0.002).Of significance, LGR5 positivity was an independent prognostic marker for better prognosis in a multivariate survival analysis adjusting for prognostic variables age, stage, gender, tumor histology and grade (HR 2.67. 95% CI 1.01-7.00, P = 0.046). DISCUSSION/SIGNIFICANCE OF IMPACT: LGR5 was significantly over expressed in colorectal cancer compared to normal tissues. LGR5 was noted to be an independent prognostic variable for an improved survival outcome in colorectal cancer patients with peritoneal metastasis, making LGR5 a potential therapeutic target in colorectal cancer patients with peritoneal metastasis.

3548

De novo germline variants in Histone 3 Family 3A (H3F3A) and Histone 3 Family 3B (H3F3B) cause a severe neurodegenerative disorder and functional effects unique from their somatic mutations

Divya R Nair[1] and Elizabeth Bhoj
[1]Children's Hospital of Philadelphia

OBJECTIVES/SPECIFIC AIMS: Histones are nuclear proteins that associate with DNA to facilitate packaging into condensed chromatin. Histones are dynamically decorated with post-translational modifications (PTMs), which regulate such processes as DNA repair, gene expression, mitosis, and meiosis. Histone 3 Family 3 (H3F3) histones (H3.3), encoded by H3F3A and H3F3B, mark active genes, maintain epigenetic memory, and maintain heterochromatin and telomeric integrity. Specific somatic mutations in H3F3A and H3F3B have been strongly associated with pediatric glia and other tumors, but no germline mutations have been reported. The goal of our study was to further understand the functional effects of germline mutations of H3F3A and H3F3B. METHODS/STUDY POPULATION: We analyzed 32 patients bearing de novo germline missense mutations in H3F3A or H3F3B with core phenotypes of progressive neurologic dysfunction and congenital anomalies, but no malignancies. Patient histones were analyzed by quantitative mass spectrometry (qMS). RESULTS/ANTICIPATED RESULTS: qMS results revealed that the mutant histone proteins are present at a concentration similar to that of wild-type H3.3. qMS analysis showed strikingly aberrant PTM patterns that suggested local dysregulation. These patterns are distinct from the dominant negative somatic mutations, which cause more global PTM dysregulation. Patient cells also demonstrated upregulation of the expression of genes related to mitosis and cell division, and had a greater proliferative capacity. DISCUSSION/SIGNIFICANCE OF IMPACT: Our data suggests that the pathogenic mechanism of germline histone mutations is distinct from that of the published cancer-associated somatic histone mutations, but may converge on control of cell proliferation. Further clarification of the pathophysiology in these patients can elucidate the roles of histones and histone PTMs in human development and non-syndromic neurodegeneration. In addition, it provides a framework for targeted therapy development for this and related progressive neurologic disorders.

3177

Determining mechanisms underlying hippocampal network disruption in early amyloid pathology

Adam Caccavano[1], P. Lorenzo Bozzelli, Katherine Conant and Stefano Vicini
[1]Georgetown - Howard Universities

OBJECTIVES/SPECIFIC AIMS: Alzheimer's disease (AD) is the leading cause of dementia, and a rapidly growing public health crisis as life expectancy increases. Two hallmark symptoms of the disease are memory impairment and the pathological accumulation of amyloid beta protein. The hippocampus is a brain region critical for the consolidation of new memories, and one of the first regions in which amyloid accumulation is observed. Our lab and others have observed a disruption to hippocampal network activity that is critical for memory consolidation in amyloid-accumulating mice. However, the mechanisms and neuronal micro-circuitry underlying this disruption are under-explored, a critical gap that warrants exploration if we are to understand memory disruption in the disease. In this study we have investigated the hypothesis that a preferential disruption to inhibitory PV neurons and the extracellular matrix that surrounds this cell type underlies downstream network alterations. METHODS/STUDY POPULATION: We have employed the 5xFAD mouse model of familial Alzheimer's disease crossed with transgenic lines that selectively fluoresce in different neuronal sub-types. In a multi-modal approach, we have investigated behavioral, electrophysiological, and biochemical alterations between 3-month-old amyloid-accumulating 5xFAD mice and littermate controls. RESULTS/ANTICIPATED RESULTS: We observe a 35% increase in the incidence of synchronous hippocampal oscillations known as sharp wave ripples (SWRs) in amyloid-accumulating mice versus littermate controls (n = 28, p = 0.01), as well as a 95% increase in the power of slow gamma oscillations (p = 0.002). This hyperexcitability of the hippocampal network is correlated with an impairment in hippocampal-dependent memory, assayed with the Barnes Maze, a behavioral test of spatial memory (172% increase in latency to find escape hole, n = 8, p = 0.01). To elucidate the micro-circuitry that

https://doi.org/10.1017/cts.2019.236 Published online by Cambridge University Press

underlies this network disruption, we have investigated the integrity of peri-neuronal nets (PNNs), part of the extracellular matrix of proteins that preferentially ensheathe inhibitory PV neurons and support their function. We observe a 60% decrease in intensity of PNNs ($n = 5$, $p = 0.005$), suggesting PNN integrity is impaired in amyloid-accumulating mice. Ongoing experiments into the activity and synaptic input to both inhibitory PV and excitatory pyramidal neurons seek to determine the effects of this PNN disruption on downstream micro-circuitry. DISCUSSION/SIGNIFICANCE OF IMPACT: These findings suggest that a preferential impairment to PNNs and inhibitory PV cells underlie hippocampal hyperexcitability in a mouse model of AD. As hippocampal network activity is critical for memory consolidation, these effects contribute to our understanding of memory disruption during early disease progression, which has been poorly understood to date. These findings provide a foundation for future in vivo studies employing optogenetic stimulation to this neuronal sub-type, to determine if restoring physiological network balance can ameliorate memory decline.

3147

Electroencephalographic suppression from anesthesia and cognitive recovery

Leonard Brian Hickman[1], ShiNung Ching, Mathias Basner, Wei Wang, Nan Lin, Max Kelz, George Mashour, Michael S. Avidan and Ben J. A. Palanca
[1]Washington University in St. Louis, Institute Of Clinical and Translational Sciences

OBJECTIVES/SPECIFIC AIMS: (1) Assess if the total duration of EEG suppression during a protocolized exposure to general anesthesia predicts cognitive performance in multiple cognitive domains immediately following emergence from anesthesia. (2) Assess if the total duration of EEG suppression in the same individuals predicts the rate of cognitive recovery in a three-hour period following emergence from anesthesia. METHODS/STUDY POPULATION: This was a non-specified substudy of NCT01911195, a multicenter investigation taking place at the University of Michigan, University of Pennsylvania, and Washington University in St. Louis. 30 healthy volunteers aged 20-40 years were recruited to receive general anesthesia. Participants in the anesthesia arm were anesthetized for three hours at isoflurane levels compatible with surgery (1.3 MAC). Multichannel sensor nets were used for EEG acquisition during the anesthetic exposure. EEG suppression was detected through automated voltage-thresholded classification of 2-second signal epochs, with concordance assessed across sensors. Following return of responsiveness to verbal commands, participants completed up to three hours of serial cognitive tests assessing executive function, reaction time, cognitive throughput, and working memory. Non-linear mixed effects models will be used to estimate the initial cognitive deficit and the rate of cognitive recovery following anesthetic exposure; these measures of cognitive function will be assessed in relation to total duration of suppression during anesthesia. RESULTS/ANTICIPATED RESULTS: Participants displayed wide variability in the total amount of suppression during anesthesia, with a median of 31.2 minutes and range from 0 minutes to 115.2 minutes. Initial analyses suggest that greater duration of burst suppression had a weak relationship with participants' initial cognitive deficits upon return of responsiveness from anesthesia. Model generation of rate of recovery following anesthetic exposure is pending, but we anticipate this will also have a weak relationship with burst suppression. DISCUSSION/SIGNIFICANCE OF IMPACT: In healthy adults receiving a standardized exposure to anesthesia without surgery, burst suppression appears to be a poor predictor of post-anesthesia cognitive task performance. This suggests that burst suppression may have limited utility as a predictive marker of post-operative cognitive functioning, particularly in young adults without significant illness.

3296

Endometrial cancer microbiome biomarker for disease detection and microbial role in the disease

Marina Walther-Antonio[1], Dana Walsh[1], Yuguang Liu[1], Janet Yao[1], Nicholas Chia[1], Heidi Nelson[1] and Andrea Mariani[1]
[1]Mayo Clinic

OBJECTIVES/SPECIFIC AIMS: Our primary objective is to determine whether the bacteria exerts its effect intra- or extra-cellularly. We have genomic and microscopy preliminary evidence indicating that the bacteria is capable of invading endometrial cells. Our secondary objective is to identify what type of impact the bacteria have on the host cells and whether they are capable of transforming the host cells from a benign into a malignant phenotype. We are currently testing a putative mechanism by which the bacteria may cause the overexpression of the hypoxia inducible factor (HIF), a hallmark of endometrial cancer. METHODS/STUDY POPULATION: We are utilizing our custom built optofluidics platform (microfluidics platform incorporated into an advanced microscope with optical laser tweezers) to isolate single cells from the endometrial tissues of 150 patients with and without endometrial cancer. We are utilizing single cell whole genome amplification followed by qPCR to identify if the bacteria is present intracellularly. We are coupling this procedure with standard microbiological invasion assays with endometrial cell line cultures and P.somerae. We are also utilizing our optofluidics platform to perform single cell transcriptomic amplification, followed by sequencing of cells invaded or in the presence of the bacteria to determine the impact in the transcriptome of the host cell. We are coupling this with western blots of factors hypothesized to be impacted by the bacteria in the overexpression of HIF. RESULTS/ANTICIPATED RESULTS: Based on our preliminary data we anticipate to find evidence that P.somerae is invading the host cells, in particular the cells in tumor tissues. We also expect to find that the intracellular presence of the bacteria is causing the overexpression of the HIF pathway, hence resulting in a cancerous phenotype. DISCUSSION/SIGNIFICANCE OF IMPACT: Our long-term goal is to develop primary prevention strategies that will reduce endometrial cancer incidence rates. A confirmation of our hypothesis could suggest that it is sufficient for endometrial cancer prevention efforts to eliminate P.somerae, in line with gastric and cervical cancer efforts. It could also mean that targeting P.somerae in cancer treatment is necessary to contain the disease and prevent recurrence.

3420

Estradiol levels are elevated in older men with diffuse cutaneous SSc and are associated with decreased survival

DeAnna Baker Frost[1], Bethany Wolf[1], Christine Peoples[2], Jessica Fike[2], Katherine Silver[1], Maureen Laffoon[2], Thomas A. Medsger Jr.[2] and Carol Feghali-Bostwick[1]
[1]Medical University of South Carolina and [2]University of Pittsburgh

OBJECTIVES/SPECIFIC AIMS: Our objective was to examine serum E2 levels in dcSSc males in relation to disease characteristics

https://doi.org/10.1017/cts.2019.236 Published online by Cambridge University Press