characterization of POMC cells during development sheds new light on the molecular diversification of early POMC neuron precursors and provides a valuable resource for elucidating the regulatory mechanisms defining POMC neuron subgroups in the hypothalamus.

Diabetes Mellitus and Glucose Metabolism

CLINICAL AND TRANSLATIONAL STUDIES IN DIABETES

Genetic Knockout of Intestinal Hexokinase Domain-Containing Protein 1 Affects Whole-Body Glycemic Control and Triglyceride Metabolism

Joseph Louis Zapater, MD, PhD, M.D. , PhDWasim Khan, MD, PhD, Brian T. Layden, MD, PhD.
University of Illinois at Chicago, Chicago, IL, USA.

MON-642

Hexokinase domain-containing protein 1 (HKDC1) is a recently discovered putative fifth hexokinase that is widely expressed in a variety of human and mouse tissues. Previous work indicate that HKDC1 is important for whole-body glucose homeostasis and utilization in pregnancy and aging, and suggest roles for HKDC1 in nonalcoholic fatty liver disease development and progression of hepatocellular carcinoma. Prior work in the lab further showed that global heterozygous-deleted HKDC1 mice exhibit blunted uptake of triglycerides following an olive oil bolus compared to wild-type mice, suggesting a role for intestinal HKDC1 expression in intestinal lipid metabolism (unpublished results). To specifically study the significance of intestinal HKDC1 on whole-body glucose and lipid homeostasis, we utilized Cre-mediated recombination of HKDC1 in which Cre was expressed under the control of the *villin* gene promoter, creating a mouse model in which HKDC1 expression is specifically deleted in the intestinal epithelium. Quantitative RT-PCR data confirmed the knockout of HKDC1 within the mouse intestine in young and aged mice, while HKDC1 expression in other tissues was comparable to wild-type mice. Next, intestinal HKDC1 knockout mice and their wild-type littermate controls were either maintained on a normal diet or were switched to a high fat diet at 6 weeks of age to simulate the state of impaired glucose tolerance, and the effects of intestinal HKDC1 on glucose and lipid homeostasis were analyzed between 28-34 weeks of age. Mice fed a normal diet did not exhibit any differences in serum glucose or triglyceride during oral/intraperitoneal glucose tolerance tests or oral olive oil bolus, respectively, regardless of intestinal HKDC1 status. Interestingly, mice lacking intestinal HKDC1 that were on a high fat diet demonstrated improved overall glycemic control compared to wild-type mice after the administration of an oral glucose load, all while there were no changes in insulin levels, gluconeogenesis or insulin tolerance related to HKDC1 status. Additionally, introduction of an intraperitoneal glucose load to mice fed a high fat diet did not alter glucose control in the presence or absence of intestinal HKDC1. However, high fat diet-fed mice lacking intestinal HKDC1 did not have a significant increase in serum triglyceride following an oral olive oil bolus, while their stool fat and triglyceride content were comparable to wild-type. Collectively, these data indicate that intestinal HKDC1 has important roles in glucose and triglyceride metabolism within the intestinal epithelium, and further suggest a role in whole-body glucose homeostasis and in the development of insulin resistance and diabetes.

Thyroid

THYROID NEOPLASIA AND CANCER

Deep-Machine Learning for Objective Quantification of Nerves in Immunohistochemistry Specimens of Thyroid Cancer

Indriani Astono, BEng (Hons)[1], Christopher W. Rowe, BSC, MBBS, FRACP[2], James Welsh, BEng (Hons) PhD[1], Phillip Jobling, BSc PhD[1].
[1]University of Newcastle, Callaghan, Australia, [2]John Hunter Hospital, New Lambton Heights, Australia.

MON-535

Introduction: Nerves in the cancer microenvironment have prognostic significance, and nerve-cancer crosstalk may contribute to tumour progression, but the role of nerves in thyroid cancer is not known (1). Reproducible techniques to quantify innervation are lacking, with reliance on manual counting or basic single-parameter digital quantification.

Aims: To determine if a deep machine learning algorithm could objectively quantify nerves in a digital histological dataset of thyroid cancers immunostained for the specific pan-neuronal marker PGP9.5.

Methods: A training dataset of 30 digitised papillary thyroid cancer immunohistochemistry slides were manually screened for PGP9.5 positive nerves, annotated using QuPath (2). 1500 true positive nerves were identified. This dataset was used to train the deep-learning algorithm. First, a colour filter identified pixels positive for PGP9.5 (Model 1). Then, a manually tuned colour filter and clustering method identified Regions of Interest (ROIs): clusters of PGP9.5 positive pixels that may represent nerves (Model 2). These ROIs were classified by the deep learning model (Model 3), based on a Convolutional Neural Network with approximately 2.7 million trainable parameters. The full model was run on a testing dataset of thyroid cancer slides (n=5), containing 7-35 manually identified nerves per slide. Model predictions were validated by human assessment of a random subset of 100 ROIs. The code was written in Python and the model was developed in Keras.

Results: Model 2 (colour filter + clustering only) identified median 2247 ROIs per slide (range 349-4748), which included 94% of the manually identified nerves. However, most Model 2 ROIs were false positives (FP) (median 85% FP, range 68-95%), indicating that Model 2 was sensitive but poorly specific for nerve identification. Model 3 (deep learning) identified fewer ROIs per slide (median 1068, range 150-3091), but still correctly identified 94% of manually annotated nerves. Of the additionally detected ROIs in Model 3, median FP rate was 35%. However, in slides where higher non-specific immunostaining was present, then the number of FP ROIs was >90%.

Conclusion: Simple image analysis based on colour filtration/cluster analysis does not accurately identify immunohistochemically labelled nerves in thyroid cancers.

Addition of deep-learning improves sensitivity with acceptable specificity, and significantly increases the number of true positive nerves detected compared to manual counting. However, the current deep learning model lacks specificity in the setting of non-specific immunostaining, which is a basis for improving further iterations of this model to facilitate study of the significance of innervation of thyroid and other cancers.

References: (1) Faulkner et al. Cancer Discovery (2019) doi: 10.1158/2159-8290.CD-18-1398. (2) Bankhead P et al. Sci Rep 2017;7(1):16878.

Adipose Tissue, Appetite, and Obesity

OBESITY TREATMENT: GUT HORMONES, DRUG THERAPY, BARIATRIC SURGERY AND DIET

Severe Copper Deficiency Post-Bariatric Surgery with Serious Preventable Complications

Asma Khaled Aljaberi, MD, Hessa Boharoon, MD.
Tawam Hospital, Al Ain, United Arab Emirates.

MON-595

Copper is an essential cofactor in many enzymatic reactions vital to the normal function of the hematologic, vascular, skeletal, antioxidant, and neurologic systems. Parenteral nutrition and chronic tube feeding are used in various malabsorptive syndromes, including following gastrectomy and gastric bypass surgery. Features of copper deficiency include hematologic abnormalities (anemia, neutropenia, and leukopenia) and myeloneuropathy; the latter is a rarer and often unrecognized complication of copper deficiency.

We describe a 36 -year-old Emirati woman who was referred to endocrinology service because of generalized body weakness and fatigue post bariatric surgery. The patient initially noted a lower extremity swelling in feet bilaterally that worsened in severity over time and progressed up to knees. Over a 3 month period, her ability to ambulate gradually deteriorated. She also noticed maculopapular skin rash over both shins. Patient had Sleeve Gastrectomy in 2011. Followed by conversion of sleeve to RYGB surgery in 2018 due weight loss failure. Patient is known to have well controlled hypothyroidism on thyroxine. She was prescribed vitamin D, neurobion, iron and multivitamins tablets post surgery but never been compliant. The patient was admitted with severe malnutrition due to poor oral intake over the last 5 months prior to admission. Her total weight loss was 34.5 kg (32% weight change, BMI 28 .52kg/m2) in less than 9 months post surgery. Initial labs revealed severe hypoalbuminemia, normochromic anemia and neutropenia. Iron, folate, thiamine, and vitamin B12 levels were normal. Vitamin B6 level was normal at 11 mcg/L (normal = 5-50 mcg/L). The serum copper level was low at 310 µg/l (normal = 794-2023µg/l). Zinc level was low at 447 µg/l (normal = 551-925µg/l). Nutritional needs were estimated using the following formulas; 22-25 kcal/kg ideal body weight (IBW)/d and 1.5-2.0 g protein/kg IBW/d, 30-35 ml IVF/kg /d. The patient's input/output, body weight, and clinical status were monitored. Parenteral nutrition additive copper 0.3 mg/day and oral copper 8 mg daily, resulted in the rapid correction of hematologic indices over one week. Combined multivitamins supplementation and oral copper supplements alone normalized serum copper levels over 4 weeks and resulted in resolution of weakness and body edema.

This report serves to alert physicians of the association between bariatric surgeries and subsequent severe copper deficiency in order to avoid diagnostic delays and to improve treatment outcomes.

Adipose Tissue, Appetite, and Obesity

NEURAL MECHANISMS OF OBESITY

The Vagus Nerve and the Hypothalamus Mediate Different Aspects of the Anorectic Effects of PYY_{3-36}

Aldara Martin Alonso, MSc[1], Simon C. Cork, PhD[1], Yue Ma, PhD[1], Myrtha Arnold, Ms[2], Herbert Herzog, PhD[3], Stephen R. Bloom, MS,DSC,FRCP,MD[1], Walter Distaso, PhD[1], Kevin Graeme Murphy, PhD[1], Victoria Salem, PhD, MMBS, MRCP[1].
[1]Imperial College London, London, United Kingdom, [2]Eidgenössische Technische Hochschule Zürich, Schwerzenbach, Switzerland, [3]Garvan Institute of Medical Research, Darlinghurst, Australia.

SAT-607

Background: Drugs that safely promote weight loss are required to treat the obesity crisis. The gut hormone peptide YY 3-36 (PYY_{3-36}) is secreted post-prandially to suppress appetite via the Y2 receptor (Y2R). However, it is unclear whether PYY_{3-36} acts directly on the Y2R in the hypothalamic arcuate nucleus (ARC) or the afferent vagus nerve to inhibit food intake. Understanding the pathways by which PYY_{3-36} mediates its anorectic effects may facilitate the therapeutic targeting of this system.

Methods: Y2R knockdown in the ARC (ARC-Y2R-KD) was achieved by stereotactic injection of Cre-expressing adeno-associated virus (AAV-Cre) in Y2R-flox C57Bl/6 mice. Y2R KD in the vagus was achieved by bilateral microinjection of AAV-Cre into the nodose ganglia (NG), where the cell bodies of vagal afferents reside. An alternative germline model of sensory nerve Y2R knockdown was generated using Nav1.8-Cre mice crossed with the Y2R-flox strain (Nav1.8-Y2R-KD). Feeding behaviour over 10 days in metabolic cages and the effects of endogenously released (after oral gavage of a nutrient bolus) or exogenously-administered PYY_{3-36} were investigated.

Results: NG-Y2R-KD animals had 60% reduction in NG Y2R mRNA but remained responsive to cholecystokinin, a positive control of vagal functionality. This is the first example of receptor specific adult vagal deafferentation in mice. The Nav1.8-Y2R-KD model achieved 30% receptor KD. Feeding patterns in the ARC-Y2R-KD and NG-Y2R-KD groups were highly different from their controls, with smaller, faster meals in the KD groups. The anorectic effects (at the next meal) of endogenous PYY_{3-36} were attenuated in NG-Y2R-KD. Low dose exogenous PYY_{3-36} at 5 µg/kg significantly reduced 2h post injection food intake (FI) in the control groups (n=8; P=0.045) but this was abrogated in the NG-Y2R-KD group. This pattern was mirrored in the Nav1.8-Y2R-KD model: low dose PYY_{3-36} significantly reduced FI 1h post-IP compared to vehicle in controls (-0.19±0.05 g; P =0.036; n=8) but not in the Nav1.8-Y2R-KD (-0.004±0.111 g; n=3). Peripherally-administered PYY_{3-36} at a high dose (30 µg/kg) decreased FI in all groups, including ARC-Y2R-KD.