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

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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oil bolus compared to wild-type mice, suggesting a role for intestinal HKDC1 expression in intestinal lipid metabolism (unpublished results).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

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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.