Title: Understanding the mechanisms of mesenteric fibrosis in small intestinal neuroendocrine tumours

Authors: Harry Hodgetts (1), Maria C. Martins (1), Marlon Lemos Dias (1), TuVinh Luong (1,2), Andrew R. Hall (1,2), Amy Webster (3), Garan Jones (3), Christos Toumpanakis (4), Dalvinder Mandair (4), Christina Thirlwell (3), Leo J. Hofland (5), Richard A. Feelders (3), Martyn E. Caplin (4) and Krista Rombouts (1)

Affiliations:

1. Institute for Liver and Digestive Health, University College London, London, UK,
2. Sheila Sherlock Liver Centre, Royal Free London NHS Foundation Trust, London, UK,
3. University of Exeter - College of Medicine and Health, Exeter, United Kingdom.
4. Neuroendocrine Tumour Unit, Royal Free London NHS Foundation Trust, London, UK,
5. Department of Internal Medicine, sector Endocrinology, Erasmus MC Cancer Institute, Rotterdam, The Netherlands

Introduction:

Mesenteric fibrosis can be a severe complication of small intestinal neuroendocrine tumours (SINETs) leading to significant complications and overall shorter patient survival. Development of new treatments is limited by a poor understanding of mesenteric fibrosis and lack of reliable SINET models.

Methods:

GOT1 cells (SINET cell line) and primary fibroblasts, isolated from normal small intestine, primary tumour, normal mesentery, and mesenteric metastases from SINET patients, were cultured together in 3D hydrogels generated using the extracellular matrix of decellularized healthy small intestine from humans. Co-cultures were analysed using Prestoblue, histology and immunohistochemistry. Bulk RNA sequencing and DNA methylation analysis was performed on a cohort of 46 SINET patients, stratified into 4 groups (none, minimal, mild, and severe) based on the severity of mesenteric fibrosis.

Results:

GOT1 cells and GOT-CAF cocultures showed good viability in human intestine ECM gels. Synaptophysin (GOT1) and αSMA (fibroblast) staining showed the distribution of cells and the localization of fibroblasts around clusters of GOT1 cells. Epigenetic and transcriptomic results were overlayed and displayed significant differences between mesenteric metastases and primary SINETs whilst also highlighting several genes with both significantly altered methylation and expression signatures. including *ACOT7*, *SFRS4* and *GNG4*.

Conclusions:

The model generated has shown promise in being able to mimic tumour cell-fibroblast interactions in a 3D structure using human ECM of the small intestine and thus, could be a better model than those currently available. The highlighted genes from analysis into patient

tissue will be taken for further analysis to understand the pathways and progression of mesenteric fibrosis. Together, these results could accelerate the research into the poorly understood mechanisms of mesenteric fibrosis and SINETs.