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**Introduction** Deficiency in the enzyme cytochrome C oxidase (CCO) has proven to be a versatile marker of clonal population in human tissues. CCO is encoded entirely by the mitochondrial DNA (mtDNA) and deficiency in CCO expression is usually attributable to mutation in the mtDNA. CCO-deficient cells are easily detectable by two-colour enzyme histochemistry. This staining provides means to identify clonal patches of CCO-deficient cells. Subsequent sequencing of mtDNA from individual cells within the patch and revealing the same somatic mutation in each cell confirms the clonality of a patch.

In the human intestine, crypts are composed of a population of a contiguous CCO-deficient mtDNA-mutated cells, and also non-mutant CCO-proficient cells are frequently observed: from the small clones occupying only a few cell positions on the crypt circumference (partially-mutated crypts), to crypts composed only CCO-deficient cells (wholly-mutated crypts). Patches of adjacent and clonal CCO-deficient crypts are also observed. Larger patches are more common in older patients indicating that CCO-deficient crypts continue to divide in the ageing colon.

**Methods** To study stem cells dynamics within intestinal crypts, we aim to characterise the shape and the size of clones in partially CCO-deficient crypts, by combining the two-colour enzyme histochemical staining with image analysis and computer reconstruction.

Multiple serial sections in the transverse plain were taken through frozen samples. Digital images were then taken of each serial section and used to create a 'crypt map' using in-house analytical software. A crypt map is a representation of the whole 3D tubular crypt unfurled and laid flat with colour enhancement post-processing.

**Results** Our results in normal and diseased human colon show that clone size can be approximated by the percentage of the crypt circumference measured from crypt transverse sections and occupied by a CCO-deficient clone.

**Conclusion** We envisage that analysis of such clonal distributions in the context of a branching process model could be used to determine the patterns of stem cell division within the human colon.

**Disclosure of Interest** None Declared.

#### PWE-158 THYMOSIN BETA 4 AS A PUTATIVE MARKER IN NEUROENDOCRINE TUMOURS

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**Introduction** Neuroendocrine tumours (NETS) arise from the diffuse endocrine system which produce biogenic amines and peptides that could be potential biomarkers. We previously analysed proteomes secreted by NET cell lines and identified mac2BP as putative marker which was also elevated in patients compared to healthy controls

**Methods** 3 Cell Lines BON-1, NCI-H727, and SHP-77 cells were grown in serum-free media overnight, which was then fractionated and the secreted 3–10kDa polypeptides were identified using Tandem Mass spectrometry. One of the small proteins, Thymosin  $\beta$ 4 was measured in serum samples of patients and controls using ELISA. Mac2BP & chromogranin A was also measured

**Results** 70 proteins were secreted by all three lines, including 20 small proteins of which 3 were thymosins  $\alpha$ 1,  $\beta$ 4 &  $\beta$ 10. Serum samples were analysed in 34 patients and 24 healthy controls. Thymosin  $\beta$ 4 was elevated in the serum of NET patients compared with healthy controls ( $p < 0.002$ ). The area under the curve was 0.84 following ROC analysis.

**Conclusion** Mass spectrometry of the secretomes of 3 NET cell lines offers a novel way of identifying potential biomarkers. Thymosin  $\beta$ 4 could be such a biomarker but further examination of tissue and other cell lines is necessary. A further analysis of serum from larger groups of patients both pre and post therapy is needed.

**Disclosure of Interest** None Declared.

#### PWE-159 HIF-1ALPHA-DEPENDENT GASTRIN GENE EXPRESSION MEDIATES RESISTANCE TO HYPOXIA-INDUCIBLE APOPTOSIS IN A HUMAN COLORECTAL CANCER CELL LINE

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**Introduction** Understanding the molecular processes mediating colorectal cancer (CRC) tumorigenesis will enable the development of targeted therapies that selectively disrupt the pathways responsible for tumour growth. The gastrin family of growth factors promote CRC growth, invasion and angiogenesis. Hypoxic microenvironments, caused by tumours outgrowing their local blood supply, stimulate aggressive tumour behaviour. However, the effect of hypoxia on gastrin expression in CRC is unknown.

**Methods** Expression of the gastrin gene in the CRC cell line LoVo was examined under conditions of normoxia and hypoxia. The effect of inhibiting expression of HIF-1 $\alpha$  (the transcriptional master regulator of cellular responses to hypoxia) and of deleting HIF-binding sites in the gastrin promoter was investigated. The effect of inhibiting gastrin expression on CRC cell behaviour *in vitro* and on tumorigenesis in mouse xenografts was analysed.

**Results** Gastrin gene expression in CRC cells is stimulated by hypoxia by HIF-1 $\alpha$  binding to the gastrin promoter. The viability of hypoxic (1% O<sub>2</sub>) gastrin knockdown cells *in vitro* is diminished due to loss of resistance against hypoxia-inducible apoptosis. In xenografts in mice exposed to hypoxia (10% O<sub>2</sub>) for 21 days, apoptosis is significantly increased by knocking down gastrin expression.

**Conclusion** This work provides evidence that gastrin expression is involved in the adaptation of CRCs to hypoxic microenvironments through resistance to apoptosis. Shrinkage of CRC liver metastases by the angiogenesis inhibitor bevacizumab is dependent on hypoxia-induced apoptosis. Therapies that target gastrin may enhance the therapeutic efficacy of bevacizumab and increase secondary resectability rates in patients with CRC liver metastases.

**Disclosure of Interest** None Declared.

#### PWE-160 INTERFERON ALPHA THERAPY FOR METASTATIC NEUROENDOCRINE TUMOURS: A RETROSPECTIVE STUDY

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**Introduction** Interferon alpha has been used in the management of NETs for over 20 years. It has generally not been popular due to perceived lack of efficacy and due to toxicity profile. Currently molecular targeted medical therapies such as mTOR inhibitors and tyrosine kinase inhibitors are promoted but studies demonstrate only modest anti-tumour effect and time to progression (TTP) with not insignificant toxicity.

**Aim** To perform a retrospective analysis of Interferon alpha (IFN $\alpha$ ) in patients with metastatic NET and assess efficacy and toxicity.

**Methods** We identified 37 patients treated with IFN $\alpha$  3–5 million units x 3 per week between 2000–2012. Mean age 58.6 (24–88) years; 26:11 male:female; 21 midgut primary, 7 pancreatic, 1 hindgut, 1 bronchial, 1 thymic and 6 unknown. Histology: G1 49%; G2 41%; G3 5%; unknown 5%. 76% were also on somatostatin analogue. 65% had recorded progressive disease at disease