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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 β4 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 α1, β4 & β10. Serum samples were analysed in 34 patients and 24 healthy controls. Thymosin β4 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 β4 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-1α (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-1α binding to the gastrin promoter. The viability of hypoxic (1% O_2) 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_2) 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 (IFNα) in patients with metastatic NET and assess efficacy and toxicity.

Methods We identified 37 patients treated with IFNα 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

onset. CT/MRI imaging; urine 5HIAA and plasma chromagranin A (CgA) and toxicities were recorded.
Results 9 (24%) withdrew before 3 months because of toxicity, progressive disease or death. On intention to treat analysis: 1 (3%) had complete response; 1 (3%) partial response; 26 (70%) had at least 3 months of stable disease. The median TTP was 14 months. Median 5HIAA fell from 54 to 29 umol/24h at 6 months (NS) and CgA from 138 to 121pmol/l at 6 months (NS). 38% had WHO grade 1–2 haematological toxicity and 19% grade 3–4. The only other grade 3–4 toxicity was depression in 1 patient and 22% had grade 1–2 depression. Other grade 1–2 toxicities > 10% included flu-like symptoms 24%, fatigue 16%, hypothyroidism 11%, dry skin 14%.
Conclusion Although there is toxicity which affects management in up to 1/3rd of patients the remaining patients tolerated therapy well. IFNα demonstrated efficacy in at least inducing or maintaining stable disease in most patients (76%). The median TTP is at least similar to other molecular targeted therapies. Those patients who were going to be intolerant or progress usually did so within the first 3 months of treatment. It would be appropriate to perform prospective randomised studies utilising IFNα and also better assess quality of life.
Disclosure of Interest None Declared.

PWE-161 SESSILE SERRATED ADENOMAS, UNDER-RECOGNISED ENDOSCOPICALLY AND UNDER DIAGNOSED PATHOLOGICALLY

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Introduction The serrated pathway of colorectal carcinogenesis is a distinct and important pathway leading to CpG island methylated phenotype (CIMP) carcinomas. These lesions are over-represented in interval cancers and may explain the failure to prevent right-sided cancer with colonoscopy. Hyperplastic polyps (HPs) in the left colon rarely transform whereas proximal serrated adenomas (SSAs) have definite malignant potential and are notoriously difficult to detect endoscopically. There is still uncertainty surrounding the diagnostic criteria and management implications of SSA's, however as pre-malignant lesions it is vital to find, resect and diagnose them. We assessed SSA diagnosis over 4 years in a teaching hospital, and assessed the (epi)genetic mutation burden and expression profile of rectal HPs versus proximal SSAs to see if identifiable molecular differences contribute to their contrasting malignant potential.
Methods We searched the pathology archives from 2009 to 2012 for the diagnosis of serrated adenomas. Cases were reviewed by 2 GI pathologists. Colonoscopic follow up for each case was assessed and compared with new American guidelines [1]. 5 distal HP and proximal SSA samples were obtained endoscopically, individual crypts were dissected and morphogen gene expression analysed. Obtained DNA was assessed for BRAF and KRAS mutation and CIMP status by methylight.
Results There were no serrated lesions diagnosed in 2009 - this was an unrecognised entity in our hospital at the time. Of 486 'hyperplastic' polyps diagnosed in 2009, 60 proximal lesions were reassessed and 19 were confirmed as SSAs. In 2010, 40 cases of SSA were diagnosed, rising to 84 in 2011 and 130 in 2012. Follow up of SSAs was appropriate in the majority of cases but lesions aberrantly denoted as hyperplastic in 2009 did not all have follow up arranged at the time. Molecular assessment showed a significant difference in (epi)mutation burden and morphogen gene expression between distal hyperplastic polyps and proximal SSA's.
Conclusion In our hospital there was no distinction made between hyperplastic and serrated lesions prior to 2010. A 3-fold increase in SSA diagnosis in the following 3 years reflects improved endoscopic detection and pathologist recognition of these lesions. The molecular difference between distal HPs and SSAs underpins the proximal predilection of CIMP cancers, and may reflect underlying differences in colonic regional microenvironment, microbiome or morphogen balance. Improved recognition of subtle endoscopic and morphological characteristics of SSAs by gastroenterologists and pathologists will improve colonoscopic surveillance.
Disclosure of Interest None Declared.

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PWE-162 CONCURRENT COMPUTERISED TOMOGRAPHY CAN OPTIMISE THE DETECTION OF CANCER IN PATIENTS PRESENTING WITH UNEXPLAINED ANAEMIA

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Introduction Active investigation for gastrointestinal (GI) cancers is often triggered by "alarm symptoms"; features in the clinical presentation that may predict malignancy and warrant urgent referral. Unexplained anaemia (UA) is a highly prevalent presentation. The BSG guidelines recommend only upper GI endoscopy (OGD) and colonoscopy (COL). We investigated the additional diagnostic value of concurrent contrast enhanced computerised tomography of the chest, abdomen and pelvis (CT) in the investigation of patients (pts) aged > 50 referred to the urgent suspected cancer (USC) pathway for GI malignancies. We evaluated its accuracy in detecting upper GI, lower GI and extraluminal malignancies in a cohort of consecutive pts presenting with and without UA.
Methods We retrospectively analysed characteristics and outcomes of 350 consecutive GI USC referrals (07/2010–07/2012): 200 (Group A) presented with UA and were investigated with OGD (178, 89%), COL (70, 39%) and CT (157, 78%, with 138, 87% aged > 50 years). The diagnostic outcomes were compared with a second group of 150 pts (Group B) referred with alarm symptoms (unintentional weight loss, abdominal pain, progressive dysphagia) who underwent OGD (91, 60%), COL (32, 21%) and CT (139, 93%, with 121, 89% aged > 50 years).
Results Group A had a mean age of 70 years (range 22–96), 51% males, mean haemoglobin (Hb) of 10.2 (5–13.8) g/dL. Pts in Group B had a mean age of 67 years (range 20–92), 60% males, mean Hb 13.9 (11.5–17.5) g/dL. Malignancy was diagnosed in 38 (19%) Group A and 17 (12%) Group B patients ($p = 0.07$). The proportion of malignant cases diagnosed endoscopically was not different across the studied groups (4/176, 2% and 7/86, 8% for OGD and COL in Group A; 5/91, 6% and 3/32, 9% in Group B, $p = 0.3$). Conversely, the rate of incident cancers identified by CT favoured Group A (33/157, 21% vs. 10/139, 7%, $p < 0.001$), where 71% of the incident cancers were extraluminal and diagnosed in pts > 50 (29/33, 87%).
Conclusion Concurrent CT can optimise the detection of malignancy in pts over the age of 50 referred under the GI USC pathway, with subjects presenting with unexplained anaemia achieving the greatest diagnostic benefit. We therefore propose that CT should be incorporated within the routine investigation pathway of anaemia in the over 50s.
Disclosure of Interest None Declared.

PWE-163 CHEMR23 AND BLT1 RECEPTOR EXPRESSION IN COLORECTAL CANCER

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