

OC-039 **EPS8: A MASTER REGULATOR OF INTEGRIN-DEPENDENT PANCREATIC CANCER INVASION**

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Introduction EGFR pathway substrate 8 (Eps8) is an adapter protein that sits at the heart of a complex system regulating re-organisation of the actin cytoskeleton. Eps8 has been shown to be involved in cell motility and EGFR internalisation, via interactions with multiple intracellular proteins, including integrins. $\alpha v \beta 6$ is an epithelial specific integrin, not present in healthy tissue but over-expressed in many carcinomas. $\alpha v \beta 6$ promotes tumour invasion and is a key activator of TGF β , which modulates tumour cell EMT and the desmoplastic response characteristically present in PC. The aim of this study was to investigate whether there is a link between Eps8 and $\alpha v \beta 6$ in pancreatic cancer invasion.

Methods We used immunohistochemistry to examine the expression of Eps8 and $\alpha v \beta 6$ in normal pancreas and PC in vivo using tissue microarrays. PC cell motility was investigated using Transwell® assays. siRNA interference was used to down-regulate genes of interest. The ability of PC cells to activate TGF β was measured using TGF β -responsive mink lung epithelial cells. Cell surface $\alpha v \beta 6$ levels were assessed with FACS analysis. Endocytosis of $\alpha v \beta 6$ was suppressed using Clathrin siRNA to examine the role of endocytosis in $\alpha v \beta 6$ functions. Pancreatic stellate cells (PSCs) were isolated from primary pancreatic resection tissue and used to optimise 3D pancreatic organotypic cultures.

Results Eps8 and $\alpha v \beta 6$ were up-regulated in > 70% PC in vivo but were not generally detected in normal pancreas. An organotypic model of pancreatic cancer was developed using primary PSCs to study $\alpha v \beta 6$ functions. $\alpha v \beta 6$ promoted PC cell invasion and TGF β activation. Interestingly, Eps8 knockdown suppressed $\alpha v \beta 6$ -dependent motility but conversely, promoted $\alpha v \beta 6$ -dependent TGF β activation. Although inhibition of $\alpha v \beta 6$ endocytosis also increased TGF β activation, this was not Eps8 dependent, nor did Eps8 knockdown affect total cell surface levels of the integrin.

Conclusion $\alpha v \beta 6$ and Eps8 are over-expressed in PC. $\alpha v \beta 6$ promotes both invasion and TGF β activation, and Eps8 appears to act as a molecular switch between these functions. How this relates to disease progression is unclear; in late stage disease, where canonical TGF β signalling is dysregulated (e.g. SMAD4 mutations which are seen in > 50% of PCs) both $\alpha v \beta 6$ functions are likely to be tumour promoting. However, in premalignancy when canonical TGF β signalling acts as a tumour suppressor, we speculate that up-regulation of Eps8 may result in a pro-invasive phenotype and facilitate tumour development. Eps8 may therefore act as a master regulator of PC invasion by switching the cell function between motility and TGF β activation at critical time points in PC progression.

Disclosure of Interest None Declared

OC-040 **WITHDRAWN BY AUTHOR****OC-041** **ENHANCED MONOCYTE FUNCTION AND SURFACE TLR EXPRESSION PREDICT POST-OPERATIVE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME IN PATIENTS IMMEDIATELY FOLLOWING MAJOR HEPATIC AND PANCREATIC RESECTION**

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Introduction Patients undergoing major surgery are at risk of systemic inflammatory response syndrome (SIRS) and life-threatening post-operative sepsis. Early identification of high-risk patients would allow for tailored post-operative care. We tested the hypothesis that post-operative SIRS could be predicted by differences in innate immune function in peripheral blood mononuclear cells (PBMC) taken from patients undergoing major abdominal surgery in the perioperative period.

Methods Serial blood samples were taken from consenting, adult patients undergoing major hepatic or pancreatic surgery pre-operatively and on days one and two postoperatively. Patients with inflammatory co-morbidities, pre-operative sepsis and those taking anti-inflammatory or immunomodulatory medication were excluded. PBMCs were isolated, stimulated for 24 hours with lipopolysaccharide (LPS) or flagellin and cytokine production was quantified by ELISA. PBMC surface expression of CD14, TLR4 and TLR5 was assessed by flow cytometry. SIRS was defined by the American College of Chest Physicians/Society of Critical Care Medicine consensus criteria.

Results We included 32 patients, nine of whom developed SIRS on day five or later. There were no significant pre-operative differences between the SIRS and the non-SIRS groups in TLR-induced IL-6 and TNF α production nor TLR4 and TLR5 expression. LPS- and flagellin- induced IL-6 concentrations were significantly greater in the SIRS group on day two (1532pg/ μ l vs 153pg/ μ l; $p < 0.0002$). Monocyte expression of both TLR4 and TLR5 was significantly greater in the SIRS group on both day one ($p < 0.002$) and day two ($p < 0.001$). There was no significant difference in CRP or other clinical predictors of SIRS between the groups in the early post-operative period.

Conclusion Patients who develop SIRS show increased cytokine production in response to TLR stimulation in the early post-operative period. This is associated with elevated surface TLR4 and TLR5 expression in monocytes. These data indicate that there are early functional differences in innate immune responses in patients who develop SIRS compared to those who do not, and that these differences are detectable days before the onset of clinical symptoms. Such assays can help to identify at-risk patients in whom intensive therapy can be targeted.

Disclosure of Interest None Declared

OC-042 **ENDOSCOPIC ULTRASOUND GUIDED FINE NEEDLE ASPIRATION OF PANCREATIC MASS LESIONS CAN BE LESS ACCURATE WITH A METALLIC STENT IN SITU BUT SHOULD NOT DELAY TREATMENT OF OBSTRUCTIVE JAUNDICE**

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Introduction Endoscopic ultrasound (EUS) examination and fine needle aspiration (FNA) of the pancreas is now a routine method of obtaining diagnostic tissue for suspected pancreatic mass lesions, however if a biliary self expanding metal stent (SEMS) is in situ it can cast an acoustic shadow and cause local inflammation making EUS visualisation and FNA interpretation more challenging. Despite this it is common for a SEMS to be in place at the time of EUS due to the early treatment required for obstructive jaundice. Conflicting results have been published regarding the success of FNA in the presence of a SEMS, our aim was to analyse the accuracy of pancreatic FNA with or without a SEMS in an hepatobiliary-pancreatic cancer network in the United Kingdom.

Methods A retrospective analysis of all FNAs performed for solid pancreatic lesions in a single centre by 2 endosonographers from August 2010 to December 2012. Patients were identified if they had a biliary SEMS or plastic stent in situ. All FNAs were performed

with 22 gauge FNA needle with no on site pathologist and direct expulsion of material into cyto-rich red medium. A standard 3 passes were performed in all lesions. Patient were excluded if they did not have a gold standard comparison for FNA results which was defined as surgical specimen comparison, death resulting from disease or clinical follow up and imaging for a minimum of 6 months.

Results 831 EUS procedure were performed during the study period of which 129 had FNA of a solid pancreatic lesions. 23 patients had a SEMS in situ at the time of EUS FNA and 5 had a plastic stent. The accuracy of pancreatic FNA with a SEMS was 65% (15/23)(95%CI: 44–81%) a plastic stent was 80%(4/5)(95%CI: 36% to 98%) and with no stent was 84.3% (86/102)(95%CI:76–90%) and overall was 82.2% (106/129)(95%CI: 75–88%). The FNA accuracy for the presence of a metallic stent was significantly lower $\chi^2 = 4.4$ ($p = 0.036$). All 8 patients with a SEMS had a false negative result of which 5 were felt well enough to undergo a further procedure for consideration of chemotherapy which gave an accuracy of 80%(4/5). All patients were happy to undergo a repeat biopsy.

Conclusion The accuracy for FNA of solid pancreatic lesions in the presence of a SEMS can be significantly lower than without and should be taken into account when consenting patients and planning treatment. However it should not delay the insertion of a SEMS as if definitive cytology is required a repeat FNA is a feasible option with an acceptable accuracy.

Disclosure of Interest None Declared

OC-043 THE ROLE OF THE EPS8 BINDING PARTNERS SOS1 AND ABI1 IN PANCREATIC CANCER

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Introduction Pancreatic cancer (PC) is an aggressive disease, characterised by marked local invasion, and identification of new molecular targets is critical to improving outcome. Cell motility requires reorganisation of the actin cytoskeleton and several actin-binding proteins have been implicated in PC. EGF receptor pathway substrate 8 (Eps8) is an adapter protein that interacts with a range of intracellular binding partners, including Abi1 and Sos1 (to form a tri-complex), and certain β integrin subunits, in order to regulate cytoskeletal reorganisation. The integrin $\alpha\beta6$ is overexpressed in approximately 70% of PC and enhances invasion. This study examines the role of the Eps8 binding partners, Abi1 and Sos1, in $\alpha\beta6$ -dependent PC invasion and the significance of their expression on patient survival.

Methods We used immunohistochemistry to examine the expression of Eps8, Sos1 and Abi1 in normal pancreas and PC *in vivo* using tissue microarrays. A retrospective patient database of PC patients operated on between 2000 and 2010 was generated. 33 short (< 1 year) and 20 long (> 5 years) survivors were then identified and resection tissue stained as whole sections for Eps8/Sos1/Abi1/ $\alpha\beta6$. We identified three PC cell lines that showed $\alpha\beta6$ -dependent invasion *in vitro*, and examined the role of Abi1 and Sos1 in Transwell® assays to specifically study motility dependent on this integrin.

Results Eps8, Sos1 and Abi1 were up-regulated in PC compared with normal tissue. Expression of these proteins in long and short survivors of PC is currently being examined. Expression of Eps8, Sos1, Abi1 and $\alpha\beta6$ were confirmed in all three PC cell lines tested. Knock-down of Eps8, Sos1 or Abi1 suppressed $\alpha\beta6$ -dependent invasion suggesting that this tri-complex is critical to PC motility.

Conclusion We have shown that Eps8, Sos1 and Abi1 are up-regulated in PC and regulate $\alpha\beta6$ -dependent function. The Eps8 binding partner Sos1 appears to be critical to $\alpha\beta6$ -dependent PC cell motility. This may be of particular interest as Sos1 expression was previously shown to fall in response to gemcitabine, the current gold standard chemotherapeutic agent for the treatment of PC. Sos1 requires further investigation as a potential molecular target in the treatment of PC.

Disclosure of Interest None Declared

Gastrointestinal physiology associates (AGIP) symposium

OC-044 EFFECTS OF SLEEVE GASTRECTOMY ON GASTRO-OESOPHAGEAL REFLUX AND OESOPHAGO-GASTRIC MOTILITY

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Introduction Sleeve gastrectomy is increasingly used as both a definitive and staggered weight loss procedure. The effect of sleeve gastrectomy on gastro-oesophageal reflux (GOR) remains uncertain. We studied the effect of sleeve gastrectomy on GOR, pressure parameters at the Lower Oesophageal Sphincter (LOS), oesophageal and gastric motility.

Methods Sixteen patients (median age 46 (range 25–71)) with morbid obesity underwent high resolution oesophageal manometry and 24hr ambulatory pH-impedance monitoring, at least 2 weeks pre-op and 3 months post sleeve gastrectomy (median no. of days post-op 129 (range 84–202)). All patients documented reflux and dysphagia symptoms at the time of testing. Nine patients also underwent concurrent gastric emptying with ¹⁵C labelled octanoate breath test. Parametric data was analysed using the paired t-test and non-parametric data with Wilcoxon matched pairs test.

Results Mean Body Mass Index (BMI) fell from 49 (41.3–58.3) to 38.5(35–46.3). 5/16 patients reported new or worsening reflux symptoms (31%). Basal LOS pressure fell from 14.4mmHg (9.7–28.5) pre-op to 8.9mmHg (0.7–40.5) post-op ($p < 0.02$). Intra-gastric pressure and G-O pressure gradient increased: 8.3mmHg (4.7–12.8) pre-op Vs 10.4 mmHg (5.3–22.7) post-op ($p < 0.01$). 8/16 patients had severe hypomotility pre-op and 9/16 post-op. 3/16 patients had pathological acid reflux pre-op, 5/16 patients having de novo reflux post-op. Mean total acid exposure time pre-op was 1.8%(0.5–5%) increasing to 4.35% (0.2–12.4%)($p < 0.02$) post-op. There was an increase in the number of acid: 18(8–31) pre-op vs 29(13–38) post-op ($p < 0.0001$), and non-acid reflux episodes: 13(7–19) pre-op vs 52(35–84) post-op($p < 0.0001$). Non-acid reflux episodes occurred predominantly in the post-prandial period. Gastric half emptying time(t1/2) was significantly shorter post-op 193.1mins (range113–433) vs 115.8 mins (range 82–170) ($p < 0.05$).

Conclusion Both acid and non-acid gastro-oesophageal reflux is increased after sleeve gastrectomy with 31% of patients developing de novo acid reflux post-op. This is despite a reduction in BMI and accelerated gastric emptying. A reduction in LOS pressure and increased G-O pressure gradient are likely to be contributing factors. Future studies should determine whether decreased gastric compliance stimulates increased numbers of transient lower oesophageal sphincter relaxations.

Disclosure of Interest None Declared