Methods From October 2010 to January 2012 (15 months) 18patients underwent laparoscopic thoracoscopic cardio-oesophagectomy. All 18 patients (12 male, 6 female) had laparoscopic insertion of Freka feeding jejunostomy are included in the study. The feeding jejunostomy was used for feeding from first post-operative day. The standard regime was water at 20 mls/h on day 1 followed by feed (jevity/osmolyte) at 30 mls/h on day 2. The rate of feed was increased at daily increments of 10 mls/h/day to achieve target rate to meet patient's nutritional requirements. Patients were discharged with feeding jejunostomy in situ, removed at follow-up if nutritionally stable.

Results The average procedure time was 20 min. Median duration of feeding jejunostomy in situ was 3 weeks (range 8 days—6 weeks). Tube related complications, n=3 patients (tube fallout-1, leak-2). Only one of these three patients needed additional parenteral nutrition. There were no procedure or feed related complications. The overall length of stay was not affected by this procedure. The availability of enteral route was useful in n=2 patients (chest infection-1, gastric stasis-1) for nutrition longer than the anticipated period.

Conclusion Laparoscopic insertion of feeding jejunostomy is safe, aids early establishment of enteral route for nutrition in patients undergoing cardio-oesophagectomy and useful in providing prolonged nutritional support in patients who develop complications were oral route is not possible.

Competing interests None declared.

Neoplasia (basic science)

PMO-088 A LARGE PROPORTION OF COLORECTAL TUMOUR-INFILTRATING CD4+ T CELLS ARE SUPPRESSIVE IRRESPECTIVE OF FOXP3 EXPRESSION

doi:10.1136/gutjnl-2012-302514b.88

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Introduction The presence of increased numbers of $CD3^+$ T cells in colorectal cancer (CRC) correlates with improved prognosis. However, it is difficult to measure anti-tumour responses in tumour-infiltrating lymphocytes (TILs) suggesting these cells are suppressed. Although we have demonstrated $CD4^+Foxp3^+$ regulatory T cells (Tregs) within the tumour and its stroma, the numbers are often low. We sought to identify phenotypic and functional characteristics of $CD4^+Foxp3^-$ T cells to determine whether other regulatory populations exist within this environment.

Methods Tumour samples were obtained from CRC patients with different stages of malignancy. Fixed tumour samples were examined by immunofluoresence for CD3, CD8 and FoxP3. TILs from fresh tumour tissue were stained with a panel of 20 antibodies (including Helios, LAG-3, LAP) and examined by FACS.

Results Histology revealed tumours to be infiltrated by $CD4^+$, $CD8^+$ and $Foxp3^+$ positive cells. Despite an increase in $CD4^+$ and $CD8^+$ T cells in advanced tumours, there was not always a concomitant increase in $Foxp3^+$ cells. Flow cytometry revealed the majority of the Treg fraction was $Helios^+$ (indicating thymically-derived) and expressed higher levels of CTLA-4 and CD39 than Tregs from colon and blood. However, 30% of "conventional" $CD4^+Foxp3^-$ T cells express markers associated with Tregs including LAP (latency-associated peptide), LAG-3 and CD25 and were highly suppressive in vitro.

Conclusion Tumour-infiltrating $CD4^+$ T cells are heterogeneous. A high percentage of these cells appear to have a regulatory function and include both $Foxp3^+$ as well as $FoxP3^-$ T cells. Overcoming the suppressive environment of CRC is a major challenge for boosting anti-tumour immunity.

Competing interests None declared.

PMO-089 PREOPERATIVE NEUTROPHIL: LYMPHOCYTE RATIO IS NOT A PREDICTOR OF OUTCOME FOLLOWING HEPATIC RESECTION FOR COLORECTAL METASTASES

doi:10.1136/gutjnl-2012-302514b.89

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Introduction A high pre-operative neutrophil:lymphocyte ratio (NLR) has been shown in several studies to be associated with shorter disease-free and overall survival for a number of malignancies, including colorectal[1] and both primary[2] and secondary[3] liver tumours. This may reflect reduced lymphocyte function, so impaired host response or neutrophil-mediated angiogenesis enhancing tumour spread and has been proposed as a prognostic predictor.[1–3] We aimed to test this association by analysing pre-operative NLR in all patients undergoing liver resection for colorectal metastases (CRM) and attempting correlation with tumour recurrence, overall and disease-free survival.

Methods Our unit is a tertiary referral centre for both laparoscopic and open hepatic surgery. A prospectively collected database of demographic details, radiological and histological findings and blood test results has been maintained since July 2005 and this data has been retrospectively analysed to demonstrate potential associations with NLR. An NLR >5 was considered raised.

Results Between 15 July 2005 and 10 January 2012 247 hepatic resections were undertaken for CRM. Median age at surgery was 67 (range 33—91) and 64% were male. Overall median survival was 1818 days and overall median disease-free survival was 542 days. 125/247 (51%) CRM developed recurrent disease within the follow-up period. Follow-up ranged from 10 days to 5.9 years (median 20 months). 30 patients had a NLR >5. When Kaplan—Meier analysis was performed to compare median survival in those with a low vs a high NLR, it was seen that there was no significant difference between the two groups (p=0.81). There was also found to be no association between NLR and tumour recurrence (p=0.49) or time to recurrence (p=0.77).

Conclusion Contrary to previously published studies, our unit has not demonstrated an association between pre-operative NLR and tumour recurrence or survival in patients undergoing liver resection for CRM and suggests that this is not a useful prognostic indicator in this group of patients.

Competing interests None declared.

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PMO-090 GALECTIN-3 INDUCES SECRETION OF CYTOKINES FROM VASCULAR ENDOTHELIUM THAT ENHANCE CANCER CELL-ENDOTHELIUM ADHESION: A NOVEL MECHANISM FOR GALECTIN-3-MEDIATED METASTASIS PROMOTION

doi:10.1136/gutjnl-2012-302514b.90

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Introduction Galectin-3 is a galactoside-binding protein whose concentration is increased up to 31-fold in the bloodstream of patients with cancer including colorectal cancer. We have recently

shown that circulating galectin-3 promotes metastasis. This effect of galectin-3 is partly due to its interaction with the transmembrane mucin protein MUC1 expressed by the tumour cells, leading to clustering of MUC1 and exposure of adhesion molecules that increases cancer cell heterotypic adhesion to vascular endothelium and cancer cell homotypic aggregation to form micro-tumour emboli. We also showed that circulating galectin-3 has another, as yet unidentified, MUC1-independent action that contributes to its promotion of metastasis.

Methods Cytokine release was assessed using a protein array that includes the 36 commonest human cytokines. Galectin-3—induced adhesion of MUC1-negative human colon cancer HCT116 and melanoma ACA19- cells to microvascular lung endothelial cells (HMVECs) were assessed.

Results The presence of galectin-3 at concentrations seen in sera of cancer patients increased the secretion of IL-6, sICAM-1, G-CSF and GM-CSF from cultured HMVECs in a galectin-3 dose- and time-dependent manner. A 117.67±13.25% ($p<0.01$), 37.84±11.89% ($p<0.05$), 100.33±14.55% ($p<0.01$) and 31.47±11.36% ($p<0.05$) increased secretion of IL-6, sICAM-1, G-CSF and GM-CSF from HMVECs to the culture medium were seen with 1 μg/ml galectin-3 after 24 hr. The culture supernatant from galectin-3-treated HMVECs increased adhesion of HCT116 (74.01±14.33%, $p<0.05$) and ACA19- (43±6.67%, $p<0.05$) cells to fresh HMVECs monolayers when compared to the supernatant from non-galectin-3 treated HMVECs. This effect was largely inhibited by the presence of a combination of neutralising antibodies against IL-6, ICAM-1, G-CSF and GM-CSF or the presence of galectin-3 inhibitor lactose. Treatment of HMVECs with galectin-3 increased the expressions of HMVEC cell surface adhesion molecules integrin$\alpha_v\beta_1$, E-selectin and ICAM-1 which was largely prevented by the presence of the four neutralising anti-cytokine antibodies in combination. Serum galectin-3 concentrations were seen to be correlated ($p=0.045$) with serum G-CSF (but not that of the other three cytokines) in colon cancer patients (n=50).

Conclusion Galectin-3, at concentrations found in the bloodstream of cancer patients, induces secretion of cytokines from the vascular endothelium that enhances cancer cell- endothelial adhesion as a result of up-regulation of the endothelial cell surface adhesion molecules. As cancer cell adhesion to blood vascular endothelium is an important step in metastasis, the secretion of those cytokines likely makes important contribution to galectin-3-mediated metastasis promotion.

Competing interests None declared.

PMO-091 TLR 9 INHIBITION: A NOVEL TARGET OF THERAPY FOR PRIMARY LIVER CANCER

doi:10.1136/gutjnl-2012-302514b.91

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Introduction Toll like receptor 9 (TLR9) is a member of the nucleotide-sensing endosomal TLR family which is critical to the innate immune defense against invading pathogens. TLR9 is activated by unmethylated CpG which is highly specific for bacterial DNA. Upon activation, TLR9 traffics from the endoplasmic reticulum (ER) to endosomes TLR9 signalling is inhibited by the aminoquinolone drug chloroquine.

Aims (1) assess changes in TLR9 subcellular distribution. (2) Detect any changes in the endolysosomal system. (3) Determine the effects on cell proliferation in hepatocellular carcinoma (HCC) and cholangio carcinoma cell (CC) lines upon stimulation and inhibition of TLR9 signalling in each case.

Methods Huh7D and HUCCT cells were treated with unmethylated *CpG (ODN 2006)* to stimulate, or chloroquine and Dynavax; IRS compound to inhibit TLR9 signalling. Cells were also treated with the TLR9 antagonist iODN. Cell growth was assessed and confocal immunofluorescence microscopy was used to determine TLR9 subcellular localisation using EEA1 and LAMP1, markers of the endolysosomal system.

Results

1. Confocal microscopy indicated a marked nuclear translocation of TLR9 in HUCCT and Huh7D when stimulated with *CpG,* while unstimulated controls showed cytoplasmic TLR9 localisation. TLR9 inhibition by iODN and chloroquine resulted in decreased cytoplasmic TLR9 meanwhile Dynavax treatment caused translocation of TLR9 to the perinuclear membranes.
2. Dramatic changes were also observed in the distribution of LAMP1 and EEA1, which were found to be localise to juxtanuclear punctae on TLR9 stimulation. While following inhibition they translocated to perinuclear membranes.
3. Huh7D cell counts the *CpG* treated cells, iODN, chloroquine and Dynavax compound were 4.5×10^5 2.1×10^5, 1.5×10^5 and 1.7×10^5 per ml respectively, compared with the untreated cells 3×10^5 per ml which indicate a significant increase in proliferation with increased TLR9 stimulation and a significant decrease with TLR9 inhibition ($p<0.03$). In HUCCT, the *CpG* treated cells, iODN, chloroquine and Dynavax were respectively 3.3×10^5, 1.8×10^5, 1.4×10^5 and 1.5×10^5 per ml compared with the untreated cells at 1.7×10^5 per ml.

Conclusion Our study indicates that TLR9 activation increases cell proliferation whereas inhibition reduces it. Our data suggest that TLR9 may be associated with tumour proliferation and may provide a potential target for therapy in liver tumours.

Competing interests None declared.

PMO-092 TLR7 EXPRESSION IS INCREASED IN HEPATOCELLULAR CANCER (HCC) AND ITS MODULATION IS ASSOCIATED WITH ALTERATIONS IN TUMOUR GROWTH: A NOVEL THERAPEUTIC TARGET

doi:10.1136/gutjnl-2012-302514b.92

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Introduction We have previously described upregulation of TLR9, which is mainly located in the endosomes, in human HCC and cell lines. Their inhibition or stimulation was associated with alteration in tumour growth. As TLR7 is also expressed on the endosomes we hypothesised that its expression may also be altered in HCC. The aim of the study was to determine whether TLR7 is expressed in human HCC and whether its modulation alters tumour growth.

Methods Study 1. Human tissue array platforms which included 102 cores of liver tissue (including 9 normal livers, 26 Hepatitis B and C, 25 HBV and HCV cirrhosis and 42 HCC) and liver tissue obtained from a DEN/NMORE model of HCC were stained for TLR7. The scoring was performed in a blinded fashion by two individual pathologists TLR7 was scored 2 when found in ≥1/3 of hepatocyte nucleus and 1 in <1/3. Study 2. Human HCC cell lines (HepG2 and Huh7) were tested for the localisation of TLR7 receptors using immunoflurocense antibody, confocal microscopy and response to stimulation was tested in the presence of a specific TLR7 agonist (Imiqumoid, Invivogen) and promega proliferation assay technique.

Results Study 1. TLR7 was expressed in the nucleus of hepatocytes in 34/42 HCC's with intense staining in 24; four out 25 positive in