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

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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

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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

cirrhosis only one showed intense staining. In HBV and HCV, 5/26 were positive and 0/9 in normal [p<0.001]. In rat tissues, TLR7 was found in all HCC tumour cells only while the background either normal, dysplastic or cirrhotic was negative. Study 2. Using confocal microscopy, TLR7 was found in the cytoplasm and the nucleus of both HepG2 and Huh7 and with stimulation of TLR7 agonist the cellular proliferation significantly increased compared to control p<0.05.

Conclusion The data show that TLR7 is highly expressed in human HCC's, animal model of HCC and in cell lines. Importantly, the background cirrhotic liver does not express TLR7. Their stimulation is associated with marked increase in proliferation. These data suggest that TLR7 may be a future target of therapy in HCC.

Competing interests None declared.

PMO-093 MRNA PROFILING OF THE CANCER DEGRADOME IN OESOPHAGO-GASTRIC ADENOCARCINOMA

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Introduction Degradation of the extracellular matrix is fundamental to tumour development, invasion and metastasis. Several protease families have been implicated in the development of a broad range of tumour types, including oesophago-gastric (OG) adenocarcinoma. The aim of this study was to analyse expression levels of all core members of the cancer degradome in OG adenocarcinoma, and to investigate the relationship between expression levels and tumour/patient variables associated with poor prognosis.

Methods Comprehensive expression profiling of the protease families [matrix metalloproteinases (MMPs), members of the ADAM metalloproteinase-disintegrin family (ADAMs)], their inhibitors [tissue inhibitors of metalloproteinase (TIMPs)], and molecules involved in the c-Met signalling pathway, was performed using quantitative real-time reverse transcription PCR in a cohort of matched malignant and benign peri-tumoural OG tissue (n=25 patients). Data were analysed with respect to clinico-pathological variables (tumour stage and grade, age, sex and pre-operative plasma C-reactive protein level).

Results Gene expression of MMP1, 3, 7, 9, 10, 11, 12, 16 and 24 was upregulated by factors greater than fourfold in OG adenocarcinoma samples compared with matched benign tissue (p<0.01). Expression of ADAM8 and ADAM15 correlated significantly with tumour stage (p=0.048 and p=0.044), and ADAM12 expression correlated with tumour grade (p=0.011).

Conclusion This study represents the first comprehensive quantitative analysis of the expression of proteases and their inhibitors in human OG adenocarcinoma. These findings implicate elevated ADAM8, 12 and 15 mRNA expression as potential prognostic molecular markers.

Competing interests None declared.

PMO-094 SUPPRESSION OF SULF2, AN EXTRACELLULAR ENDOSULFATASE UP-REGULATED IN HEPATOCELLULAR CANCERS, MODULATES WNT SIGNALLING AND INHIBITS CELL GROWTH

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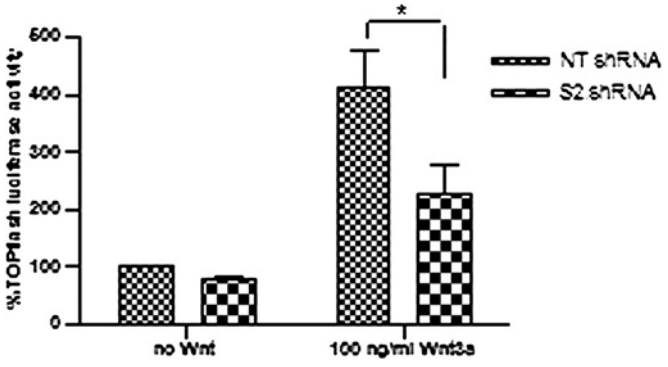
Introduction Hepatocellular carcinoma (HCC) is the 3rd most common cause of cancer death globally and effective systemic treatments for the disease are limited. HCC complicates chronic liver disease and its incidence is increasing dramatically in the UK. Sulfatase 2 (SULF2) is one of two extracellular heparan sulphate 6-*O*-endosulfatase and one of 17 human sulfatases. It reportedly modulates ligand activated FGF and Wnt signalling and is up-regulated in 57% of HCC. We aim to explore the potential of SULF2 as a therapeutic target for HCC treatment and have characterised its biology in HCC cell lines.

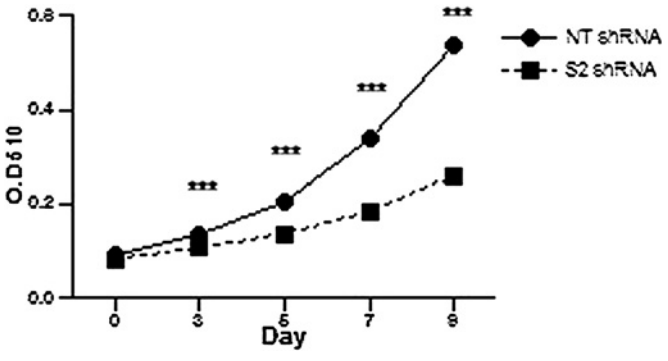
Methods Expression of SULF2 and its homologue SULF1 were assessed at RNA and protein levels in six HCC cell lines. The desulfating enzymatic activity of these cell lines were compared using the fluorogenic substrate 4-methylumbelliferyl sulphate (4-MUS). SULF2 was knocked down using short hairpin RNA lentiviral particles. SULF2 gene silencing effect on receptor tyrosine kinase signalling was investigated by phospho-ERK and phospho-AKT immunoblot and its effect on Wnt signalling by the TCF luciferase reporter assay. Cell growth was assessed by SRB assay.

Results 3 of the six tested HCC cell lines showed up-regulated SULF2 expression at the RNA and protein levels. HuH-7 cells had the highest sulfatase activity. SULF2 gene silencing in this cell line caused dramatic inhibition of Wnt3a-induced β-catenin-dependent transcriptional activity (twofold and p value = 0.03, Abstract PMO-094 figure 1), with relatively modest effects on the phosphorylation of ERK or AKT after stimulation with FGF-1, FGF-2 or IGF-I. SULF2 suppression significantly reduced cell number (twofold and p value <0.0001, Abstract PMO-094 figure 2) and enzymatic activity (p value <0.0001, Abstract PMO-094 figure 3) of HuH-7 cells.

Conclusion SULF2 is over-expressed in the majority of HCCs and is catalytically active. SULF2 gene silencing in HuH-7 inhibits Wnt signalling and cell growth. These data support a key role for SULF2 in hepatocarcinogenesis, the inhibition of which offers a novel means of antagonising Wnt signalling in cancers.



Abstract PMO-094 Figure 1 SULF2 knockdown inhibits Wnt signalling.



Abstract PMO-094 Figure 2 SULF2 knockdown decreases sulfatase enzymatic activity.