INVESTIGATION OF IL-33 SERUM LEVELS IN PATIENTS WITH BENIGN AND MALIGNANT SALIVARY GLAND TUMOURS

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Introduction Interleukin-33 (IL-33) is a member of the IL-1 family of cytokines that has a dual function which acts both as an extracellular alarming cytokine and an intracellular nuclear factor. Previous studies have shown that IL-33 play important role in induction of type-2 immune responses. In addition, this cytokine is intrinsically expressed in epithelial barrier tissues to maintain barrier function in normal conditions, and is released as a danger signal immediately after cellular damage or stress. Moreover, IL-33 helps tumour to evade from immune responses by promoting expansion and immunosuppressive effect of myeloid-derived suppressor cells.

Material and methods 42 patients with malignant salivary gland tumours including mucoepidermoid carcinoma (MEC), adenoid cystic carcinoma (ADCC), acinic cell carcinoma, squamous cell carcinoma, malignant mixed tumour and 14 patients with pleomorphic adenoma (PA) as benign salivary gland tumour cases were enrolled in this study. Also, 28 healthy subjects were matched as the control group. Serum levels of IL-33 was measured by sandwich ELISA method.

Results and discussions The mean concentrations of IL-33 in malignant, benign and healthy subjects were 6.73±2.97 pg/ml, 5.47±2.52 pg/ml and 5.26±5.89 pg/ml, respectively, which were significantly different (p=0.001). The serum levels of IL-33 were significantly increased in ADCC (8.07±4.59 pg/ml) and MEC (6.93±1.60 pg/ml) malignant salivary gland types compared with PA (5.47±2.52 pg/ml), (p=0.05 and p=0.009 respectively) and the control group (5.26±5.89 pg/ml) (p=0.003 and p=0.001 respectively). There were also positive and significant correlations between stage (p=0.005) and tumour size (p=0.024) of salivary gland tumour patients with IL-33 concentration.

Conclusion According to our results, IL-33 could be suggested as a novel biomarker used to distinguish different types of salivary gland tumours.

GENETICAL INHIBITION OF IRE1α IN MACROPHAGES SUPPRESSES HEPATOCELLULAR CARCINOMA GROWTH

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Introduction The microenvironment of hepatocellular carcinoma (HCC) will be synchronised to tumor-promoting niche by the cytokine and growth factors produced by infiltrated inflammatory cells. Macrophages, in particular, have been regarded as the inflammatory effector in HCC. Inositol-requiring enzyme 1α (IRE1α) is an unfolded protein response sensor, that controls protein regulation, metabolism and inflammation. Inhibition of IRE1α has been reported to inhibit metabolic inflammation through macrophages modulation. Therefore, the aim of this study is to investigate the role of IRE1α in tumour associated macrophages of HCC.

Material and methods Bone marrow isolated mononuclear cells were differentiated by either culturing with M-CSF or tumour supernatant for 7 days. Expressions of mRNAs was detected by quantitative real time PCR; protein expression was detected by immunoblotting assay; gene silencing was performed by RNA interference. HCC proliferation was detected by BrdU incorporation assay. The animal model was established by implanting subcutaneous grown tumour cube to left lobe of mouse liver. The hepatic tumour growth was monitored by in vivo luciferase imaging system.

Results and discussions We observed pharmacological inhibition of IRE1α by genipin, an iridoid aglycone reduced expressions of inflammatory factors such as CCR2, TNFα, CCL5, iNOS and IL6 on tumour supernatant-cultured and M-CSF induced macrophages. LPS-induced inflammatory gene expression in macrophages was similarly suppressed by genipin treatment. The role of IRE1α was confirmed from the observation of suppression of all the inflammatory mediators in IRE1α silencing macrophages. Furthermore, co-incubation of HCC cells with IRE1α silencing macrophages inhibited HCC cells proliferation. Our further in vivo study showed suppression of orthotopic HCC tumour growth after genipin intervention. We observed reduced IRE1α expression in hepatic macrophages after genipin treatment. This was accompanied with consistent reduction of inflammatory markers on the sorted hepatic macrophages.

Conclusion Altogether, our findings unveil the role of IRE1α in modulating inflammation in macrophages and pharmacological or genetical inactivation of IRE1α in macrophages suppressed HCC progression.

DISSECTING THE ROLE OF REGULATORY T CELLS IN METASTATIC BREAST CANCER

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Introduction Breast cancer is the most frequent malignancy among women worldwide. More than 90% of breast cancer-related deaths are due to metastatic disease. Despite these facts, breast cancer metastasis remains incurable. A key player in metastasis is the immune system. Cancer-induced immunosuppression contributes to a tumour’s ability to evade immune destruction. A major cell type in this process is the regulatory CD4+ T cell (Treg). It has been reported that Tregs are found in the microenvironment of primary tumours and metastases, and that their depletion reduces metastatic burden; however, the underlying mechanisms remain poorly understood. Using state-of-the-art breast cancer mouse models that closely recapitulate primary human breast cancer and metastatic disease, our goal is to elucidate the impact of Tregs on breast cancer metastasis, focusing on differences between (pre-)metastatic tissues and different steps in the metastatic cascade.

Material and methods To study primary spontaneous mammary tumours and the pre-metastatic niche, we primarily use the FVB K14cre; Cd1d1F/F; Tp53F/F (KEP) conditional mouse model for invasive lobular carcinoma. For metastatic disease, KEP tumour fragments are orthotopically transplanted into in wild-type syngeneic FVB mice. Following tumour outgrowth and
mastectomy, widespread metastatic disease is present in lungs, lymph nodes and other distant organs, providing an accurate representation of the different steps of the metastatic cascade.

**Results and discussions** We observed systemically elevated levels of Tregs prior to metastatic disease. These tumor-educated Tregs display a distinct phenotype and specifically accumulate in the lung and lymph node metastases that arise in our metastasis models, perhaps indicating their possible importance for metastasis formation and progression.

In addition to these systemic changes, within primary tumours and metastases a large population of PD1\textsuperscript{high} Tregs is found. Preliminary data suggest that tumor-associated myeloid cells influence this population.

**Conclusion** We are currently setting out to dissect the mechanism behind this interplay of intra-tumoral immune cells and the role of distinct Tregs found prior to metastatic disease.

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**PO-387 PHENOTYPIC AND FUNCTIONAL ANALYSIS OF MALIGNANT MESOTHELIOMA TUMOR-INFLTRATING LYMPHOCYTES (TILS)**

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**Introduction** Given the growing interest and promising preliminary results of immunotherapy in mesothelioma, it has become important to more fully understand the immune landscape in this tumour. This may be especially important for deciding who might benefit most from checkpoint blockade or agonist antibody therapy. Although the phenotype of tumour infiltrating lymphocytes (TILs) in mesothelioma is being increasingly well described, there is very little functional analysis of mesothelioma TILs.

**Material and methods** Fresh mesothelioma tissue and blood were sampled from patients enrolled in an on-going clinical trial of radical pleurectomy vs. radical pleurectomy and photodynamic therapy (PDT) at our institution. Following processing, single cell suspensions were used for phenotypic using flow cytometry. Functionality of TILs was assessed by measurement of cytokine (IFN-\(\gamma\) production) following overnight anti-CD3 antibody stimulation ex vivo. These data were compared to TILs from early stage lung cancer patients.

**Results and discussions** Flow cytometric analysis of 19 patient samples so far shows that the numbers of CD8-positive TILs are markedly low at around 3\%–10\% of total live cells. TILs have an effector memory phenotype enriched for CD103 + tissue memory cells, they show high expression of inhibitory receptors PD-1 (25\%–80\%) and TIGIT (50\%–80\%), and have an Eomes\textsuperscript{high} T-Bet\textsuperscript{low} phenotype. Unlike TILs from early stage lung cancers, which are generally able to make cytokines, TILs from advanced stage mesotheliomas show profound hypofunction in their ability to produce IFNo (<20\%), which is our readout for TIL function. Cells expressing high levels of the transcription factor EOMES and the surface marker CD28 seem especially dysfunctional.

**Conclusion** Our results show that TILs from mesothelioma tumours are profoundly hypofunctional. Areas of active interest are to determine the mechanisms and best markers of T cell hypofunction. Such information might allow us to understand with TILs might be activated by checkpoint inhibitors. We also plan to correlate T cell function with clinical responses in our trial.

**PO-388 THE GASTROINTESTINAL TRACT TUMOUR MICROENVIRONMENT DIFFERENTIALLY INFLUENCES MATURATION OF AND CYTOKINE SECRETION FROM DENDRITIC CELLS**

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**Introduction** Oesophageal adenocarcinoma (OAC) and rectal adenocarcinoma are treated with neoadjuvant chemoradiotherapy in order to reduce tumour size prior to surgery however only 10\%–30\% of patients have a complete pathological response. Inflammatory and angiogenic mediators in the tumour microenvironment (TME) have many functions, such as enabling evasion of anti-tumour immune responses by disabling infiltrating dendritic cells (DCs) and have been linked with radioresistance. Tumour Conditioned Media (TCM) from colonic cancer has been shown to strongly inhibit DC maturation. Our aim was to understand if this DC inhibition extends to other cancers of the gastrointestinal tract, to investigate if radiotherapy influences this and to profile constituents of TCM that may influence DC maturation.

**Material and methods** TCM from 0Gy or 2Gy-irradiated cell lines or tumour biopsy explants, was used to pre-treat monocyte-derived DCs prior to stimulation with LPS to measure DC maturation based on DC cell surface markers (HLA-DR, CD86, CD54, CD80, CD83 and PD-L1) and two cytokine levels (IL12 p70 and TNF alpha). Inflammatory and angiogenic mediator multiplex ELISAs were used to profile the TCM of oesophageal and rectal adenocarcinoma.

**Results and discussions** DCs remained responsive to LPS following pre-treatment with OAC cell line TCM, whereas extensive inhibition was induced by CRC cell line TCM. ex vivo TCM from different gastrointestinal adenocarcinoma types induced different effects on DC maturation with oesophageal inducing DC activation, rectal inducing minor activation and colonic inducing inhibition of DC maturation markers. Interestingly, all cancer types induced DC inhibition of secreted TNF alpha. It was also found that 2Gy-irradiated TME induced significant inhibition of DC maturation for irradiated rectal adenocarcinoma and no effect with irradiated oesophageal cancer. Differential levels of inflammatory (IL2) and angiogenic mediators (Ang2 and bFGF) in TCM of GI tumours correlated with DC maturation.

**Conclusion** Overall, this study offers new evidence that there are differences in the human TME from different gastrointestinal (GI) cancers which can directly induce varying levels of inhibition of LPS-induced DC maturation markers, whilst all inhibit secreted TNF alpha.