# Gut – IDDF Young Investigator Award
# Basic gastroenterology

IDDF2020-ABS-0073 | **PROTEOMICS REVEALS THAT RECTAL CANCER PATIENTS WITH NEOADJUVANT RADIOCHEMOTHERAPY REACH PCR THROUGH IMMUNE ACTIVATION**

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**Background** Colorectal cancer (CRC) is the second leading cause of cancer death worldwide, and more than 1/3 of all cases are rectal cancer. The standard neoadjuvant radiochemotherapy for locally advanced rectal cancer fails to benefit all patients due to individualize sensitivity to radiotherapy. It's critical to understand the molecular mechanisms underlying pathological complete regression (pCR) in some patients.

**Methods** We collected 67 patients with rectal cancer who were treated with long-term radiotherapy and capecitabine chemotherapy from two hospitals. Among them, a total of 58 cases with both pre-treatment endoscopic biopsy specimens and surgical pathological sections available were picked procured and reassessed for Tumor Regression Grade (TRG) after treatment mentioned above. Formalin-fixed paraffin-embedded (FFPE) tissue samples from each individual were collected with two biological replicates. All the samples were processed by Pressure Cycling Technology coupled with Data-Independent Acquisition mass spectrometry for proteomic profiling. The abundances of immune infiltrates and their correlation with CDH11 were estimated by TIMER algorithm.

**Results** A total of 6483 proteins are quantified with high confidence with a high Pearson correlation ($R^2=0.98$). Fifty-eight patients were divided into two groups according to the pCR condition after neoadjuvant radiochemotherapy. At the threshold of the adjusted p-value of 0.05 and fold change of > 1.5, we identified 127 up-regulated proteins and 205 down-regulated proteins in the pCR group. The former proteins were mainly involved in immune response and cell activation, while the latter mostly participated in metabolic processes. TIMER algorithm suggested a higher degree of immune cell infiltration in the pCR group, especially involving CD8+ T cell and Dendritic cells. The significantly up-regulated protein, Cadherin-11 (CDH11), was identified as a key factor contributing to various immune infiltrates, including T cells, Macrophage and Dendritic cells. Mutation of CDH11 gene has a high correlation between its copy number variation (CNV) and abundance of immune infiltrates. More details will be presented.

**Conclusions** Based on the proteomic analysis of biopsy specimens before neoadjuvant therapy, immune activation was identified as the potential mechanism via which some rectal cancer patients attained pCR.

IDDF2020-ABS-0110 | **LNCRNA MNX1-AS1 PROMOTES THE PROGRESSION OF COLORECTAL CANCER THROUGH STABILIZING YB1**

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**Background** Improving the prognosis of Colorectal Cancer (CRC) depends on the identification of the mechanisms of recurrence and metastasis, and developing new therapeutic targets. lncRNA has become an attractive potential therapeutic target because it is more precise and less toxic when compared with traditional protein targeted drugs. We aimed to identify novel lincRNAs that significantly affects the development of CRC, and investigate the potential associated therapeutic targets.

**Methods** Based on RNA-seq analysis, we screened out ten candidates and identified highly expressed lncRNAs in the 21 CRC tissue samples. An siRNA library was established to identify lncRNAs that significantly affected CRC cell proliferation and metastasis. RT-PCR and RNAi assays were performed to investigate the functional role of lncRNA MNX1-AS1 and clinical relevance. In vivo, cell-based and patient-derived xenograft (PDX) models were used to further explore roles of MNX1-AS1 in CRC tumorigenesis, metastasis and potential therapeutic target. RNA pull-down, mass spectrometry analyses, western blot and RNA-binding protein immunoprecipitation (RIP), DNA-binding protein immunoprecipitation (Chip), and Double Luciferase Report experiment were performed to identify interaction proteins and related mechanisms.

**Results** MNX1-AS1 was upregulated in CRC tissues from patients with poor overall survival (OS), and MNX1-AS1 inhibition led to the impaired CRC cell line growth. Moreover, knockdown of MNX1-AS1 resulted in a decreased level of Y-box binding protein 1 (YB1), a multifunctional RNA/DNA binding protein. MNX1-AS1 blocked ubiquitination of YB1 and maintained its stability. This process prevented the degradation of YB1 through the MYC pathway. Therefore, knockdown of MNX1-AS1 attenuated the downstream effects of YB1. In addition, the transcription of MNX1-AS1 could be inhibited by MYC in CRC cells. In vivo experiments showed that the inhibition of MNX1-AS1 suppressed the proliferation of tumors in orthotopic models and patient-derived xenograft (PDX) models.

**Conclusions** The newly identified MNX1-AS1, which is regulated by MYC, plays a pivotal role in CRC proliferation by enhancing YB1 stability, thus facilitating the development of CRC. Collectively, our study suggests that MYC- MNX1-AS1-YB1 axis might serve as potential biomarkers and therapeutic targets in CRC treatment.

IDDF2020-ABS-0118 | **LONG NON-CODING RNA LRTIS REGULATES MLL1-MEDIATED IMMUNOCHECKPOINT REMODELLING IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA**

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**Background** Tumor immune evasion is an important hallmark of cancer. The roles of long non-coding RNAs (lncRNAs) in the process of cancer immunosurveillance remain largely unknown. In this study, we aimed to identify oncogenic lncRNAs that are involved in the immunosuppression of esophageal squamous cell carcinoma (ESCC) and investigate underlying mechanisms.

**Methods** High-throughput sequencing and bioinformatics analysis were used to identify lncRNAs that are highly expressed in ESCC tissues and blood. Using the data from a cohort of patients in the clinical trial JSOO1, the relationship between lncRNA expression level and PD-1 mAb response (ORR and DCR) was analyzed. RNA interference and CRISPR-Cas9 were used to explore the functional roles of the lncRNA. In vivo, the human PBMC engrafted humanized xenograft model was established to assess the therapeutic responses of that specific lncRNA inhibitor and its combination with PD-1 mAb.

**Results** Increased expression of LRTIS was observed in ESCC and was correlated with poor prognosis. Conversely, LRTIS high expression group responded better to immunotherapy (ORR 19.56% vs 9.09%, DCR 54.35% vs 18.18%). In vivo, LRTIS knockout significantly inhibited tumor growth in Hu-PBMC mice, and increased sensitivity to PD-1 mAb treatment, as shown by an increased proportion of IFN-γ+CD8+ T cells in xenografts. LRTIS could inhibit the expression of immuno-checkpoints such as PD-L1, PD-L2 and IDO1. Mechanistically, LRTIS was directly associated with MLL1 and significantly affected MLL1 stability by inhibiting its ubiquitination and subsequent proteasomal degradation. LRTIS competitively bonded to MLL1 preventing ASB2 from binding to MLL1, which explained MLL1 ubiquitination inhibition. Knocking out LRTIS, resulted in MLL1 mediated H3K4me decrease in the promoter region of immuno-checkpoints, causing their down-regulation. Clinically, dysregulation of LRTIS-MLL1-PD-L1 axis could also be observed in patients.

**Conclusions** The LRTIS plays an essential role in ESCC immunosuppression by binding to and stabilizing MLL1. This study identified a novel immuno-checkpoint regulating lncRNA and revealed a novel mechanism underlying lncRNA-mediated cancer immuno-microenvironment remodeling. Translational studies further implicated that LRTIS is a promising prognostic biomarker for cancer immunotherapy, and a potential target for immunotherapy.

## Basic hepatology

IDDF2020-ABS-0166  **NOVEL MIRNA-BASED DRUG CD5–2 REDUCES LIVER TUMOUR GROWTH IN DIETHYLNITROSAMINE (DEN)-TREATED MICE BY NORMALISING TUMOUR VASCULATURE AND ALTERING IMMUNE INFILTRATE**

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**Background** Hepatocellular carcinomas (HCC) exhibit abnormal (leaky) vasculature, hypoxia and an immunosuppressive microenvironment. The normalisation of tumour vasculature is an emerging approach to treat many cancers. Blockmir CD5-2 is an oligonucleotide-based inhibitor of the miR-27a interaction with VE-Cadherin, the endothelial specific cadherin. We previously showed CD5-2 normalises tumour vasculature by increasing VE-Cadherin expression (Zhao et al. Cancer Res. 2017). We studied the effect of CD5-2 combined with checkpoint inhibition on liver tumour growth, vasculature and immune infiltrate in the DEN-induced mouse model.

**Methods** DEN was given (25 mg/kg intraperitoneally) to male C57BL/6 mice at postnatal day 14. CD5-2 (30 mg/kg intravenously fortnightly) and/or anti-PD1 antibody (250μg intraperitoneally every 4 days) with their respective controls (4 groups) were given to the mice from age 7-months until harvest at age 9-months. Livers from treated and untreated mice were analysed.

**Results** We analysed human HCC data from The Cancer Genome Atlas and found high miR-27a and low VE-Cadherin were both associated with poorer survival (Log-Rank $P=0.02$ and $P=0.01$, respectively). In untreated mice, miR-27a
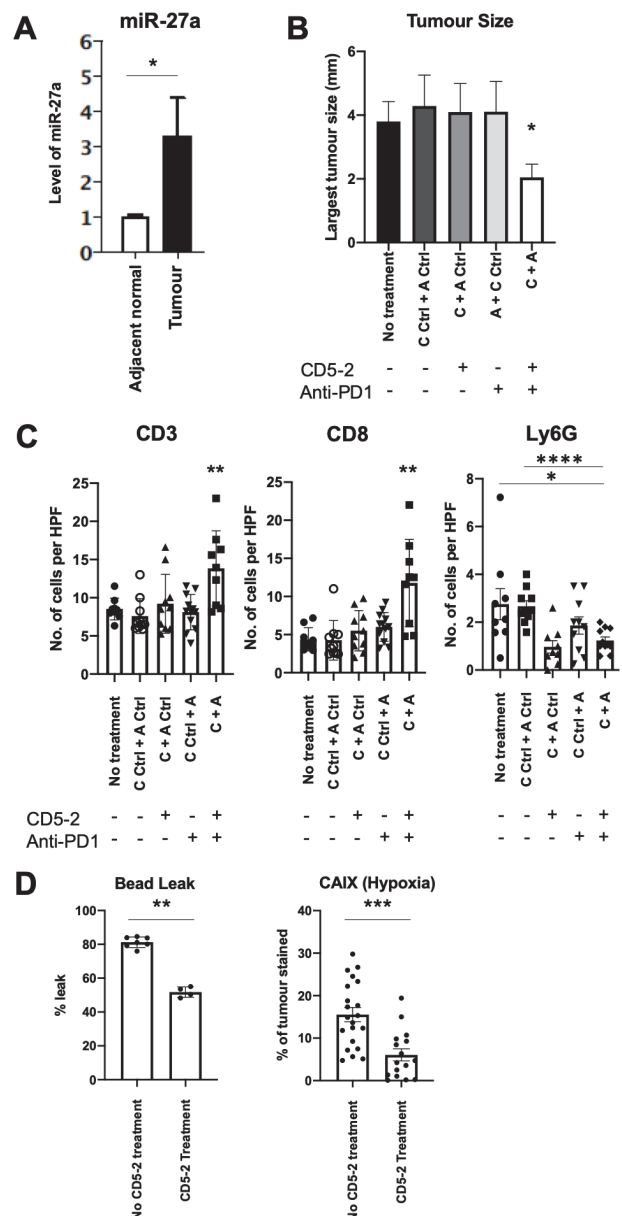
Abstract IDDF2020-ABS-0166 Figure 1