HCC and gastric cancer. It is more important that 61 gene mutations in the pancreatic cancer data set do not coincide with the other two datasets.

Among them, KRAS is located on the chromosome 12 P12.1, gene type is a protein-encoding gene. The rate of mutation in pancreatic cancer is 137/163 (84.05%). The number of mutant species is 13. The total survival rate of KRAS mutations in 162 cases of pancreatic cancer was significantly lower than that no mutation in KRAS (p=1.18e-2). SCN5A is located on the chromosome 3 p22.2, gene type is a protein-encoding gene. The rate of mutation in pancreatic cancer is 10/163 (6.13%). The number of mutant species is 15. The total survival rate of SCN5A mutations in 162 cases of pancreatic cancer was significantly lower than that no mutation in SCN5A (p=2.99e-2).

**Conclusions** There is a significant correlation between the KRAS and SCN5A gene mutations and the prognosis of pancreatic cancer.

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**RECURRENT GENE AMPLIFICATION SIGNATURE ASSOCIATED WITH INTRAHEPATIC METASTASIS OF HEPATOCELLULAR CARCINOMA**

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**Background** About 50%–75% of hepatocellular carcinoma (HCC) cases present with multiple intrahepatic foci in the liver at diagnosis, with poor prognosis and limited therapeutic options available. In this study, we aimed to identify recurrent genetic alterations of multifocal HCC.

**Methods** Whole genome sequencing and RNA sequencing were performed in 12 tumours and six matched non-tumour liver tissue samples to screen recurrent alterations in multifocal tumours. The selected genes were then analysed using data from The Cancer Genome Atlas (TCGA). Their biological functions were explored by *in vitro* experiments. Clinical impact of targeted genes was assessed in 60 patients with multifocal HCC in our medical centre.

**Results** ZNF687, ANXA9 and RABIF were identified as top candidates for their copy number gain and upregulated transcriptional activity in 10 tumours. The mRNA expression of these genes was upregulated in tumour tissues, with a positive correlation with gene amplification in 370 HCC cases from TCGA. Functional studies of ZNF687, ANXA9 and RABIF revealed that they could significantly increase cell proliferation and migration. Furthermore, the protein expression of ZNF687 was significantly higher in tumour tissues as compared with their adjacent normal tissues and overexpression of ZNF687 was significantly associated with shortened recurrence-free survival and overall survival in patients with multifocal HCC.

**Conclusions** We have identified three recurrent genetic alterations of multifocal HCC - the amplification and overexpression of ZNF687, ANXA9 and RABIF, which were highly associated with intrahepatic metastasis. Our findings may inspire innovative therapeutic approaches for multifocal HCC.

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**IRRERSIBLE ELECTROPORATION INDUCES IMMUNOGENIC CELL DEATH IN HEPATOCELLULAR CARCINOMA AND PROMOTES SYSTEMIC ANTI-TUMOUR IMMUNE EFFECTS IN MICE MODELS**

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**Background** The therapeutic efficacy of ablation on hepatocellular carcinoma (HCC) is impeded due to high tumour recurrence. The immune response induced by ablation has provided new insights into tumour monitoring and elimination. Irreversible electroporation (IRE) ablates tumours through high frequency electric pulse in a non-thermal manner, which could preserve more intact tumour antigens and provide a more powerful anti-tumour immune response theoretically. We aimed to explore the effects of immune reaction mediated by IRE.

**Methods** *In vitro*, characteristics of HCC cell death were determined by trypan blue staining, flow cytometry (FCS) and transmission electron microscope analysis. Immunogenic cell death (ICD) was detected by calreticulin or HSP70 exposure by FCS, ATP secretion by luciferase and DC maturation assay. *In vivo*, C57BL/6 mice were employed to establish tumour ablation model, in which dynamics of immune infiltration were analysed by FCS and immunohistochemistry. The adaptive immune response was further confirmed by CD8 blockade, and vaccine experiments were performed.

**Results** We found that IRE ablation could effectively result in HCC cell death via necrosis and induced positive molecular determinants of ICD including increased ecto-CRT and ecto-HSP70 exposure and elevated extracellular ATP level. In addition, the dendritic cells co-cultured with IRE-induced tumour lysis were activated evidenced by upexpression of co-stimulators and increased secretion of cytokine. Using mice model, we found IRE significantly inhibited tumour growth accompanied by increased CD8 +IFN-γ +T cells and reduced PD-1 +cells and Treg. Depletion of CD8 +T cells abolished the therapeutic effect of IRE that local tumour recurrence and distant metastasis were promoted. IRE-based vaccine experiments showed vaccinated mice significantly resisted secondary tumour induction and demonstrated a long-term immunological memory response.

**Conclusions** Our study revealed that IRE treatment trigged ICD, enhanced immune response upon tumour recurrence and distant metastasis through CD8 +T cells and induced alleviation of immunosuppression.

**IDDF2018-ABS-0139**

**METALLOTHIONEIN 1G IS SILENCED BY DNA METHYLATION AND CONTRIBUTES TO THE PATHOGENESIS OF HEPATOCELLULAR CARCINOMA**

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**Background** The therapeutic efficacy of ablation on hepatocellular carcinoma (HCC) is impeded due to high tumour recurrence. The immune response induced by ablation has provided new insights into tumour monitoring and elimination. Irreversible electroporation (IRE) ablates tumours through high frequency electric pulse in a non-thermal manner, which could preserve more intact tumour antigens and provide a more powerful anti-tumour immune response theoretically. We aimed to explore the effects of immune reaction mediated by IRE.

**Methods** *In vitro*, characteristics of HCC cell death were determined by trypan blue staining, flow cytometry (FCS) and transmission electron microscope analysis. Immunogenic cell death (ICD) was detected by calreticulin or HSP70 exposure by FCS, ATP secretion by luciferase and DC maturation assay. *In vivo*, C57BL/6 mice were employed to establish tumour ablation model, in which dynamics of immune infiltration were analysed by FCS and immunohistochemistry. The adaptive immune response was further confirmed by CD8 blockade, and vaccine experiments were performed.

**Results** We found that IRE ablation could effectively result in HCC cell death via necrosis and induced positive molecular determinants of ICD including increased ecto-CRT and ecto-HSP70 exposure and elevated extracellular ATP level. In addition, the dendritic cells co-cultured with IRE-induced tumour lysis were activated evidenced by upexpression of co-stimulators and increased secretion of cytokine. Using mice model, we found IRE significantly inhibited tumour growth accompanied by increased CD8 +IFN-γ +T cells and reduced PD-1 +cells and Treg. Depletion of CD8 +T cells abolished the therapeutic effect of IRE that local tumour recurrence and distant metastasis were promoted. IRE-based vaccine experiments showed vaccinated mice significantly resisted secondary tumour induction and demonstrated a long-term immunological memory response.

**Conclusions** Our study revealed that IRE treatment trigged ICD, enhanced immune response upon tumour recurrence and distant metastasis through CD8 +T cells and induced alleviation of immunosuppression.
Background Primary hepatocellular carcinoma (HCC) is one of the most common malignancies all over the world. However, the mechanism of HCC initiation and development remains unclear. In our previous work, high-throughput microarray assay in HCC samples followed by bioinformatic analysis suggested that Metallothionein1G (MT1G) might be one of the key factors in HCC. In this study, we aim to clarify the biological function of MT1G and validate its potential to be utilised as a biomarker in HCC.

Methods We detected the MT1G expression in paired HCC samples and cell lines by both RT-qPCR and Western blot. MSP (Methylation specific PCR) and BGS (Bisulfite genomic sequencing) were performed to evaluate methylation status of MT1G in HCC. TCGA (The Cancer Genome Atlas) data analysis was used to validate the results from our samples. The functional significance of MT1G was investigated by overexpression or knockdown in vivo and in vitro. Kaplan-Meier survival analysis was performed using TCGA data to estimate the clinical value of MT1G expression and methylation status in HCC.

Results MT1G was inactivated in 4 of 6 HCC cell lines. The expression of MT1G was downregulated in cancer tissues compared with the adjacent non-tumour tissues (p<0.001). The gene expression of MT1G was closely correlated to the promoter methylation status. The MT1G expression in silenced HCC cell lines could be restored by demethylation agent. Ectopic re-expression of MT1G by stable transfection in SMCC7721 and Hep3B cells inhibited colony formation (p<0.001), suppressed cell motility and invasiveness (p<0.05), accompanying with up-regulation of E-cadherin; and down-regulation of PCNA, MMP2, MMP13 and Vimentin. Xenograft tumour assay in nude mice also revealed that MT1G could markedly decrease tumour weights and volumes in vivo (p<0.001). Survival analysis revealed that hypermethylation of MT1G predicts good outcomes of HCC patients.

Conclusions Our results demonstrate that MT1G promoter methylation directly mediates the transcription down-regulation and commonly occurs in HCC. MT1G gene can act as a functional tumour suppressor in liver carcinogenesis by playing an important role in the depression of cell proliferation, migration and invasion.

IDDF2018-ABS-0165 CIRCULAR RNA CIRC3739–6 PERFORMS FUNCTIONS IN INHIBITING TUMORIGENESIS AND METASTASIS OF HEPATOCELLULAR CARCINOMA VIA THE REGULATION OF PPARα

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Background Circular RNAs (circRNAs) are considered as RNA molecules in loop structure and was reported as a potential novel biomarker for hepatocellular carcinoma (HCC). Peroxisome proliferator-activated receptor alpha (PPARα) is a suppressor of HCC via direct targeting nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IkBα) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signalling pathway according to our previous study, whereas their relationship in the modulation of pathogenesis processes in HCC is still unclear. The aim of this study was to determine the circRNAs those perform functions in regulating the gene PPARα and thereby regulate the pathogenesis processes in HCC.

Methods The candidate circRNAs were firstly validated in structure via the RNase R treatment, amplification of specific convergent and divergent primers, and Sanger sequencing. After over-expressing the circRNAs in HCC cell lines and further validation of the cell viability assay, the circRNA correlated to PPARα was obtained. Functional analysis of the levels of 5-methylcytosine (5-mc). Western-blot and IF assays were used to detect the protein levels of DNA methyltransferases (DNMTs) and RT-PCR was performed to test transcription of DNMTs. Cycloheximide was used to suppress the protein translation. Moreover, MG132 and BafA1 were used to inhibit the functions of ubiquitin-proteasome and autophagy-lyosome respectively to evaluate the effect of posttranslational modification for DNMTs degradation. Finally, we selected twenty liver cancer cell lines and combined western-blot and CCK8 assays to observe the association between mTORC1 inhibition-induced DNMTs degradation and cell viabilities.

Results Rapamycin treatment reduced 5 mC levels in HCC cell lines. In parallel, mTORC1 inhibition attenuated the expressions of DNMT1. The subsequent experiments revealed that transcriptional regulation and posttranslational modification is not likely to participate in the process of DNMT1 degradation induced by mTORC1 inhibition. However, cycloheximide half-life assay indicated that mTORC1 might adjust the expression DNMT1 through regulating initial translational efficiency. We found that mTORC1-mediated DNMT1 degradation is closely linked to cell proliferation.

Conclusions In conclusion, we demonstrated that mTORC1 could affect DNA methylation levels in HCC probably through regulating DNMTs expression. Besides, this interactive role seems to be closely linked to HCC cell viability. Investigation of the relationship between mTORC1 signalling and DNA methylation will deepen our insight on the pathogenesis of HCC and develop a novel therapeutic strategy for HCC patients.