IDDF2018-ABS-0117 IDENTIFICATION OF MOLECULES DRIVING HEPATIC METASTASIS FORMATION OF GASTRIC CANCER CELLS

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Background Hepatic metastasis and relapse contribute to the high incidence of gastric cancer (GC)-related fatalities and represent a frequent and crucial problem for oncologists. The aim of this study was to identify and explore diagnostic biomarkers and molecular targets to predict and treat hepatic metastases.

Methods Transcriptome and bioinformatics analyses were conducted using tissues from patients who had GC with synchronous hepatic metastasis to identify a candidate molecule that specifically mediates hepatic metastasis of GC. Stable knockout cells were established by genome editing using the CRISPR-CAS9 system. *In vitro* analyses including proliferation, apoptosis, migration, invasion and adhesion assays were performed to evaluate the effect of knockout. Mouse subcutaneous xenograft and liver metastasis models were used to evaluate influences of knockout on tumorigenicity and formation of hepatic metastasis. mRNA levels of the candidate molecule were determined in the gastric tissues of 200 patients with GC to assess whether differential gene expression predicted patient prognosis.

Results Synaptotagmin VII (SYT7) was identified as a candidate biomarker for hepatic metastasis in GC. GC cell lines exhibited differential SYT7 expression levels and the mRNAs encoding SNAI1 and TGFB3 was expressed at levels that correlated significantly with those of SYT7 mRNA, whereas the expression levels of RGS2 mRNA correlated inversely with those of SYT7. The proliferation, invasion, adhesion and migration abilities of stable SYT7-knockout GC cells were significantly reduced compared to MKN control cells. Stable SYT7-knockout GC cells showed significantly more annexin V positive cells in comparison to the MKN1 cells. Tumorigenicity of GC cells in mouse subcutaneous xenograft models was significantly decreased in stable SYT7-knockout GC cells accompanying reduced Bcl-2 and HIF-1a protein expression in subcutaneous tumours. Tumour growth after portal injection of cells was significantly decreased in stable SYT7-knockout GC cells. High SYT7 expression in clinical GC tissues was associated with shortened overall and disease-free survival.

Conclusions SYT7 represents a promising target for treatment of hepatic metastasis of GC.

IDDF2018-ABS-0121 INNOVATIVE MASS SPECTROMETRY PROBE VIA POLARITY-REVERSAL DERIVATIZATION FOR MAPPING GLOBAL CARBOXYL-CONTAINING METABOLITES IN COLORECTAL CANCER HUMAN SERUM

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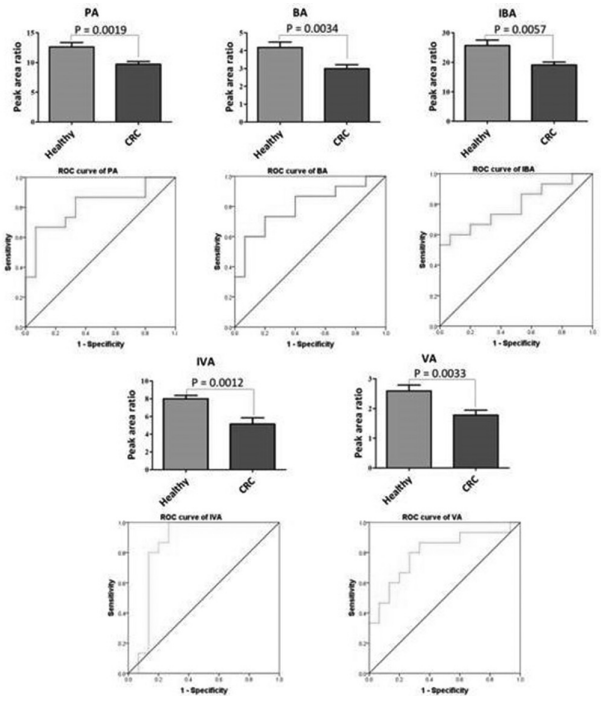
Background Carboxyl-containing metabolites (CCMs) widely exist in the living system and are the essential components of life. Global characteristics of CCMs in biological samples represent a challenge due to enormous polarity difference, structural diversity, high structural similarity, and poor ionisation efficiency in mass spectrometry.

Methods An innovative mass spectrometry probe, 5-(diisopropylamino)amyline (DIAA), was developed for mapping the profile of CCMs using derivatization-liquid chromatography-mass spectrometry.

Results With this innovative methodology, the sensitivity was enhanced up to 25,000-fold. Moreover, the hydrophobicity of polar CCMs, amino acids, TCA cycle intermediates and short-chain fatty acids, and the hydrophilicity of low-polar CCMs, long-chain fatty acids and bile acids, were significantly increased, resulting in a remarkable separation efficiency for which 68 different CCMs can be simultaneously determined. The polarity-reversal was revealed to be induced by the different impacts of aliphatic chains and nitrogen atom in DIAAA, the latter existing as a cation in the acidic mobile phase. Furthermore, a novel isotope labelled probe, DIAA-d_{14}, was also synthesised and utilised to develop an isotope labelling method based on DIAA/DIAA-d_{14} pair for quickly and accurately hunting for the potential biomarkers in colorectal cancer (CRC), which is the second leading cause of death in the world. Finally, 52 CCMs were detected in the sera of CRC patients and 28 CCMs related with amino acids metabolism, TCA cycle, fatty acid metabolism, and gut flora metabolism were found to have a significant difference with that in healthy controls. The decrease of five SCFAs, including propionic acid (PA), butyric acid (BA), isobutyric acid (IBA), isovaleric acid (IVA) and valeric acid (VA) in the sera (figure 1). It was further confirmed in line with that of $Apc^{Min/+}$ mice, a well-established animal model of intestinal cancer.



Abstract IDDF2018-ABS-0121 Figure 1

Conclusions These results clearly indicated a close relationship of the change of serum SCFAs with CRC for the first time.

This innovative mass spectrometry probe via polarity-reversal derivatization was confirmed to be a valuable tool for metabolomics study with high sensitivity and separation efficiency in various biological samples.

IDDF2018-ABS-0122 MICRORNA EXPRESSION ANALYSIS OF ADVANCED COLORECTAL CANCER REVEALS A MICRORNA SIGNATURE WITH PROGNOSTIC AND PREDICTIVE VALUE

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Background Prognostic and predictive markers are needed to predict the clinical outcomes of patients with advanced colorectal cancer (CRC) who receive standard first-line treatments. We performed a prospective cohort study in advanced CRC patients to identify a miRNA signature that could predict the benefit of receiving first-line chemotherapy for these patients.
Methods Twenty-one paired tumours and adjacent normal tissues were collected from advanced CRC patients and analysed by miRNA microarrays. Between tumours and normal tissues, 33 miRNAs were differentially expressed and was confirmed by qRT-PCR from another group of 67 patients from a prospective cohort study. A two-miRNA-based signature was obtained using the LASSO Cox regression model based on the association between the expression of each miRNA and the PFS of individual patients. Internal and external validation cohorts, including 40 and 44 patients with advanced CRC, respectively, were performed to prove the prognostic and predictive value of this signature.
Results A signature was built based on two miRNAs, miR-125b-2–3 p and miR-933. CRC patients were classified into low- and high-risk groups for disease progression based on this tool. The patients with low-risk scores generally had better PFS than those with high-risk scores. In the training set, the median PFS in the low- and high-risk groups were 12.00 and 7.40 months, respectively. In the internal validation set, the median PFS in the low- and high-risk groups were 9.90 and 5.10 months, respectively. In the external validation set, the median PFS in the low- and high-risk groups were 9.90 and 6.40 months, respectively. Furthermore, we detected miR-125b-2–3 p associated with CRC cell sensitivity to first-line chemotherapy.
Conclusions Our two-miRNA-based signature was a reliable prognostic and predictive tool for tumour progression in patients with advanced CRC and might be able to predict the benefit of receiving standard first-line chemotherapy in CRC.

IDDF2018-ABS-0125 OVEREXPRESSION OF LNCRNA LINC00460 AFFECTS CELL PROLIFERATION AND APOPTOSIS BY REGULATING KLF2 AND CUL4A EXPRESSION IN COLORECTAL CANCER

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Background Emerging evidence has proven that long noncoding RNAs (lncRNAs) play important roles in human colorectal cancer (CRC) biology, while few lncRNAs have been characterised in CRC. Therefore, the functional significance of lncRNAs in the malignant progression of CRC still needs to be further explored.
Methods By utilising publicly available lncRNAs expression profiling data and other publicly available lncRNAs expression profiling data, we screened out LINC00460, whose expression is significantly increased in CRC. The quantitative reverse transcriptase PCR (qRT-PCR) was used to analyse the expression of LINC00460 in 60 CRC tissues and correspond adjacent normal tissues and four CRC cell lines. Gain and loss of function approaches were used to investigate the biological role of LINC00460 both *in vitro* and *in vivo*. Bioinformatics analysis followed by qRT-PCR was performed to identify the putative targets of LINC00460, which were further verified by RNA immunoprecipitation (RIP), Chromatin immunoprecipitation (ChIP), Luciferase reporter assays, rescue experiments and western blotting assays.
Results We found a novel lncRNA, LINC00460, whose expression was significantly over-expressed in all three publicly available microarray data. Consistently, qRT-PCR results also verified that LINC00460 was over-expressed in CRC tissues and cells. Furthermore, high LINC00460 expression levels in CRC specimens were correlated with larger tumour size, advanced tumour stage, lymph node metastasis and shorter overall survival. *In vitro* and *in vivo* assays of LINC00460 alterations revealed a complexly integrated phenotype affecting cell growth and apoptosis. Mechanistically, LINC00460 repressed kruppel like factor 2 (KLF2) transcription by binding to the enhancer of zeste homolog 2 (EZH2). LINC00460 also functioned as a molecular sponge for miR-149–5 p, antagonising its ability to repress cullin 4A (CUL4A) protein translation.
Conclusions Taken together, our findings support a model in which the LINC00460/EZH2/KLF2 and LINC00460/miR-149–5 p/CUL4A crosstalk serve as critical effectors in CRC tumorigenesis and progression, suggesting new therapeutic directions in colorectal cancer.

IDDF2018-ABS-0126 THE PSEUDOGENE DUXAP8 PROMOTES PANCREATIC CANCER CELL PROLIFERATION AND INHIBITES CELL APOPTOSIS BY EPIGENETICALLY SILENCING CDKN1A AND KLF2

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Background Pseudogene has been shown to involve in human cancer biology, but their mechanisms of action are mainly undocumented. Current evidence suggested that pseudogene play a critical role in the regulation of pancreatic cancer cellular processes, such as proliferation, apoptosis, and metastasis. However, only a small proportion of these pseudogene has been functionally characterised.
Methods By utilising publicly available pseudogene expression profiling data and other publicly available pseudogene expression profiling data, we screened out DUXAP8, whose expression is significantly increased in pancreatic cancer (PC) tissues.