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CHARACTERIZATION OF CHI3L1 AS A POTENTIAL PLASMA BIOMARKER FOR ENDOSCOPIC RESECTABLE GASTRIC CANCER BY SWATH-MS AND TRANSCRIPTOME DATABASE ANALYSIS

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Background Early diagnosis of T1a gastric cancer (GC) provides patients opportunities for endoscopic minimally invasive resection, which avoids the trauma of surgery and improve quality of life. However, most recent biomarkers were discovered from early/advanced mixed GC patients, which showed limited potential in identifying endoscopic curable GC patients.

Methods The overall study design was shown in figure 1A(figure 1A). Here we recruited 5 T1aN0M0 GC patients received endoscopic resection of GC and 5 age/sex-matched chronic superficial gastritis (CSG) controls. Plasma samples were collected before endoscopic resection or any other treatment. We used SWATH-MS proteomics to screen for up-regulated proteins in GC plasma, and the detailed workflow was shown in figure 1B (figure 1B). Then we identified differentially expressed genes (DEGs) of five GC datasets by GEO2R to construct a consensus list of up-regulated genes in GC. Overlapped secreted/membrane proteins between this consensus list and SWATH-MS up-regulated list were verified in an independent cohort by ELISA.

Results We identified 37 up-regulated and 21 down-regulated proteins in GC plasma by SWATH-MS, which could well distinguish GC from CSG. Ten of those proteins were antibody fragments, which could not be mapped to a single gene. The rest 48 genes were associated with response to stress, extracellular space, and ion binding, according to GO analysis. For online database analysis, 174 genes were identified as DEGs in all those databases, but most of them were down-regulated. There were 94 genes up-regulated in at least 3 databases, and most of those genes were mutually associated in a PPI network. 58 of the 94 genes were secreted or membrane-associated, and only 1 gene, CHI3L1, was characterized as up-regulated in both the database consensus list and the SWATH-MS list. Finally, ELISA in an independent cohort (n=42) verified that CHI3L1 was significantly higher in the plasma of T1a GC than that of healthy controls ($p<0.001$).

Conclusions We provide a novel strategy for biomarker screening combining recent MS technique with public database analysis, and identified plasma CHI3L1 as a potential biomarker for endoscopic resectable GC patients

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LACTOBACILLUS RHAMNOSUS GGSUPERNATANT IMPROVES BOWEL FUNCTION VIA UPREGULATING 5HT4R AND MUC2 EXPRESSION AND MODULATING MICROBE ENVIRONMENT IN MICE

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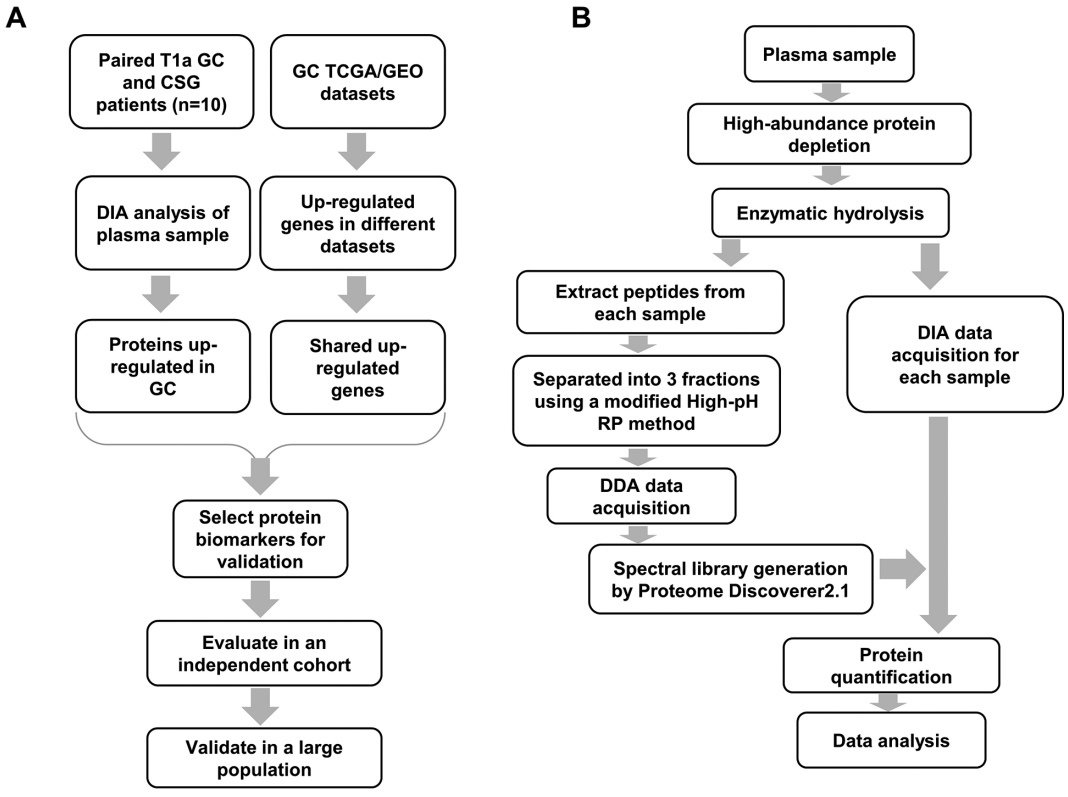
Background *Lactobacillus rhamnosus GG* (*LGG*) has been reported to improve bowel function in constipation patients.



Abstract IDDF2019-ABS-0297 Figure 1 A) General analysis pipeline; B)

However, the effects and mechanism of *LGG* on bowel function remains unclear. 5-Hydroxytryptamine4 Receptor is a critical receptor relating to the intestine motility and secretion function. In this study, we aimed to investigate whether *LGG* could improve the defecation function *via* upregulating 5-HT4R and modulating gut microbiota in mice.

Methods Male C57BL/6 mice 6–8 weeks in age were randomly divided into 3 groups: MRS group (n=10), Tegaserod group (positive control, n=15) and *LGG* group (n=15), and MRS broth, tegasromide maleate and *LGG* supernatant were gavaged respectively for 7 days. YAMC cells and Caco2 cells were used for experiment *in vitro*. Defecation parameter including the number of pellets in 2 hours, fecal weight, fecal dry weight, fecal water content, and the gastrointestinal transit time (GITT) were detected. PAS and AB-PAS staining were used to evaluate goblet cells number in mice colon, and 5-HT4R and MUC2 expression were determined Real-time PCR and Western blotting *in vivo* and *in vitro*. Gut microbiota and short-chain fatty acid were analyzed by 16 sRNA pyrosequencing analysis and gas chromatography method.

Results The number of defecation pellets in 2 h, fecal weight, fecal dry weight and fecal water content in the Tegasromide group and *LGG* group were significantly increased compared with those in the MRS group, PAS staining showed that the average number of goblet cells in Tegasromide group and *LGG* group were significantly increased in mice colon sections compared with MRS group. AB-PAS showed increased cavitated goblet cells in the *LGG* group, and the mRNA and protein levels of 5-HT4R and MUC2 were upregulated both *In vitro* and *in vivo*. In this study, increased levels of *Alistipes*, *Allobaculum*, and *Desulfovibrioin* were found in the *LGG* group which have been reported to be involved in intestine motility, intestinal barrier.

Conclusions *LGG* supernatant could improve defecation function in mice accompanied by upregulating 5-HT4R and MUC2 production, and modulating gut microbiota. Thus, this study will provide a better understanding of probiotics for the prevention and treatment of constipation.

IDDF2019-ABS-0301 MAPPING OF GUT MICROBIOME SECRETOME IN COLORECTAL CANCER: A MALAYSIAN DATA

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Background The human gut is home to trillions of gut flora that thrive in a delicate balance, which has helped maintain the host's gut homeostasis and mutually benefited both parties tremendously. However, a drastic perturbation of microbial composition has hampered gut homeostasis initiating tumour microenvironment for the development of colorectal cancer (CRC). The objective of this study was to profile secreted proteins released from the human gut and microbial of CRC patients and control with healthy colon morphology by assessing the secretome in stool samples, using mass spectrometry technology.

Methods Stool samples from 26 CRC and 20 controls were collected, homogenized and filtered prior to protein extraction and analysis. Samples were subjected to in-solution digestion, followed by protein identification and quantification. Bioinformatics tools such as SPSS, MaxQuant, DAVID and String were used for statistical analysis, data visualization, functional annotations and prediction of protein interactions and pathways.

Results We identified more human origin proteins in CRC as compared to control & inversely for proteins from microbial origin. The identified human exclusive proteins for CRC were mostly related to protein binding function and the top expressed proteins were mapped to Stage I and II CRC. The best prediction model was built upon the combination of human Huntingtin & RNA exonuclease 5 proteins. The model was sensitive but not specific in discriminating control from CRC. Meanwhile, the top annotated KEGG pathway for human CRC-exclusive proteins was Hypoxia-inducible factor-1 (HIF-1). In addition, yeast proteins were topping the microbial CRC-exclusive proteins list, with the predicted protein interactions mapped to DNA repair, transcription regulation & ATP binding.

Conclusions In conclusion, gut flora and human colon released an abundance of microbial proteins to the external environment possibly mediating various host-microbial reactions and responses in CRC.

IDDF2019-ABS-0303 METHYLATION PROFILES OF PHANTOM 5 ENHANCER AND OPEN CHROMATIN IN COLORECTAL CANCERS

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Background Colorectal cancer (CRC) contributes around 1.36 million of the total cases worldwide and it has become evident over the past two decades that epigenetic alterations also play key roles in CRC pathogenesis. Majority of the research epigenetic alterations has examined the promoter regions, while other loci such including enhancers and open chromatins are not yet well described. Hence, this study aims to specifically profile the methylome of enhancers and open chromatins in CRC

Methods Genomic DNA and total RNA were extracted from cancer-adjacent normal colonic tissues and subjected to bisulfite conversion and cDNA synthesis, respectively. DNA methylation analysis was performed using Human Infinium Epic Beadchip Array which includes >23,000 enhancers and >461,000 open chromatins. Microarray data were analyzed using Genome Studio V1.8 and Bioconductor-ChAMP V2.8.1. The differentially methylated regions were validated via bisulfite conversion, cloning, and sequencing of individual clones. In order to correlate the effect of DNA methylation at the specific loci, the gene expression of the differentially methylated loci was analysed using quantitative real-time PCR

Results We identified 342 significant differently methylated enhancers and 2187 significant differentially methylated open chromatin. There were 192 hypermethylated and 150 hypomethylated enhancers compared to 1110 hypermethylated and 1076 hypomethylated open chromatins. Pathway enrichment