Category: AMED P-CREATE **Poster Number: 1**

Title: Initial clinical trial for practical use of early diagnosis marker of colon cancer.

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Abstract:

The current screening method for colorectal cancer (CRC) is fecal occult blood test and blood CEA value measurement in Japan, however, in both cases the accuracy of the diagnosis is too low to detect early colorectal cancer.

It is technically very difficult to screen markers contained in blood at low abundant level, thus we have performed proteomic screening using extracellular vesicles (EVs) in blood instead of using whole blood. We discovered sensitive and specific 37 CRC early detection markers using MS-based SRM assay. AUC values of two single peptides in annexin family proteins were over 0.96. Furthermore, we found AUC of these-marker peptides were over 0.91 and 0.98 when stage 1 patients and stage 2 patients were compared with healthy control respectively in the second cohort.

We will also report the latest results of verification of the tumor specificity of our early-diagnosis CRC markers.

Category: AMED P-CREATE Poster Number: 2

Possibility for Efficient Pancreatic Cancer Screening Using a Blood Test for Apolipoprotein A2-Isoforms

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For developing the screening method of pancreatic cancer, an efficient enrichment strategy using blood testing in high-risk individuals (HRIs) before imaging is important. We identified a unique alteration in apolipoprotein A2 isoforms (apoA2-i) that have different C-terminal amino acids in pancreatic cancer and its precancerous lesions. We developed an ELISA kit to measure circulating apoA2-i concentrations in peripheral blood as a tool for efficient pancreatic cancer screening.

We prospectively measured apoA2-i in serum samples collected from participants enrolled in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. We performed ELISA measurements of CA19-9 and apoA2-i in 156 patients with pancreatic cancer and 217 matched controls using plasma samples collected up to 60 months prior to diagnosis. In addition, we carried out experimental pancreatic cancer screening with the apoA2-i blood test for 5,120 participants in Kobe.
In a prospective evaluation by the EPIC study, joint models based on ApoA2-i plus CA19-9 improved the sensitivity of prediagnostic samples of pancreatic cancer. At 98% specificity, and for lag times of ≤6, >6–18 or ≤18 months, sensitivities were 57%, 36% and 43% for CA19-9 combined with ApoA2-i, respectively.

Eighty-four of 5,120 participants enrolled in Kobe underwent experimental screening using the apoA2-i blood test as positive cases (positivity rate = 1.6%). Fifty-four of 84 participants underwent imaging examination; pancreatic abnormalities were detected in 26 participants, including 1 case of pancreatic adenocarcinoma and 9 cases of IPMNs. The positive predictive value of pancreatic abnormality including HRI was 33.3%.

The results of our study demonstrate that the apoA2-i blood test can be used as an efficient enrichment strategy for HRI by non-invasive blood testing before imaging.

Category: AMED P-CREATE **Poster Number: 3**

Prediction of Cancer Tissue of Origin by Circulating Mirna Profiles

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Abstract

The ultimate goal of blood-based cancer tests is early-stage detection with the information of tissue of origin. In Japan, a national project entitled “Development and Diagnostic Technology for Detection of miRNA in Body Fluids” was conducted between 2014 and 2019. This project includes the comprehensive characterization of serum miRNA profiles of 13 types of human cancers, such as breast, lung, stomach, colorectal, esophagus, liver, pancreatic, biliary tract, prostate, bladder, and ovarian cancers, sarcomas, and gliomas, in more than 40,000 of preserved serum samples using highly-sensitive microarray system (3D-Gene, Toray). Using this huge dataset, we created a diagnostic system that accurately classifies cancers based on the serum miRNA profiles using an advanced machine learning algorithm, Hierarchical Ensemble Algorithm with Deep learning (HEAD) collaborated with Preferred Networks, Inc.. After training using miRNA microarray data from 7931 serum samples obtained from patients with 13 types of solid cancers and 5013 non-cancer samples, we tested 1190 cancer and 1256 non-cancer samples. HEAD predicted cancer tissue of origin with an overall accuracy of 0.92, significantly better than simpler machine learning algorithms. This demonstrated that the established machine learning can extract hidden features of tissue-specific miRNA alterations and represents a highly promising blood-based early cancer classification.

Category: AMED P-CREATE **Poster Number: 4**

Identification of Glioblastoma-selective Secreted Proteins by Secretome Analysis, and their CSF levels in GBM patients

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Glioblastoma (GBM) is known as a malignant brain tumor, and GBM biomarkers are required to detect cancer at an earlier stage for improving therapeutic outcomes. However, GBM biomarkers possessing the potential for clinical applications have not been identified yet. The purpose of the present study was to identify GBM-selective secreted proteins from the conditioned-mediums (CMs) of GBM cells and validated their expression in the cerebrospinal fluid (CSF) of GBM or non-brain tumor patients.

GBM (U87, U251, T98G) and non-GBM (MDA-MB-231, MCF-7, Caco-2) cells were cultured with FBS-free medium. The proteins in CMs were identified by data-independent acquisition. The identified proteins were quantified by targeted proteomics with spiking internal-standard peptides.

We identified 2,371 proteins from the CMs, and 1,338 proteins were identified by more than 3 peptides. Among 1,338 proteins, 19 proteins were detected only in the CMs of GBM cells. To validate the GBM-selective secretion, the expression levels of 19 proteins were quantified in the CMs and CSF by targeted proteomics. As a result, 19 proteins exhibited higher expression levels in CMs of GBM cells than those of non-GBM cells. The expression of laminin subunit alpha-4 (LAMA4) and osteopontin (OPN) were significantly greater in CSF of 22 GBM patients than in 11 non-brain tumor patients and showed AUC greater than 0.86. Therefore, LAMA4 and OPN were suggested to be secreted from GBM tissues. In conclusion, LAMA4 and OPN were identified as GBM-selective secreted proteins, and are candidates as biomarkers and therapeutic targets for GBM.

Category: AMED P-CREATE **Poster Number: 5**

Global Proteomic Analysis of Viable Tissue-Derived Exosomes and Identification of Exo-CAT1 as a Potential Early Detection Marker for Colorectal Cancer

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Abstract

Exosomes are nano-scaled vesicles secreted from any types of cells into various body fluids. Especially, cancer cell-derived exosomes are considered to have a great potential as biomarker carriers. In this study, we performed global proteomic profiling of exosomes secreted from viable CRC tissues.

The tissue-exudative extracellular vesicles (Te-EVs) were obtained from serum-free culture media of freshly resected viable CRC tissues or adjacent normal mucosa (n = 17). We analyzed these Te-EV samples and also original tissue samples by Orbitrap Fusion Lumos with FAIMS-Pro LC/MS system (Thermo Scientific) and performed label-free quantification analysis for detected 6,307 Te-EV proteins and 8,565 tissue proteins.
In addition to identification of a new class of exosomal luminal protein marker “VPS family”, cationic amino acid transporter 1 (CAT1) was found to be loaded specifically on CRC-derived exosomes ($p = 5.0 \times 10^{-3}$, fold-change = 6.2).

The exosome sandwich ELISA assay using 119 plasma samples (94 CRC patients and 25 healthy donors) showed that expression level of exosomal CAT1 (Exo-CAT1) in CRC patients’ plasma was significantly higher than that in healthy donors’ plasma (AUC of ROC curve = 0.821). Importantly, Exo-CAT1 was clearly upregulated in Stage-I CRC patient group ($p = 2.0 \times 10^{-6}$).

Further metabolomic analysis revealed that CAT1-overexpressed EVs drastically enhanced vascular endothelial cell growth and tubule formation via upregulation of arginine transport and downstream NO metabolic pathway (Ikeda, A., *et al.*, Mol Cancer Res, 2021).
These findings demonstrate the potency of CAT1 as an exosome-based biomarker for colorectal cancer and its functional significance on tumor angiogenesis.

Category: AMED P-CREATE **Poster Number: 6**

Circulating Cancer-Associated Extracellular Vesicles As Early Detection And Recurrence Biomarkers For Pancreatic Ductal Adenocarcinoma

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Abstract

Extracellular vesicles (EVs) attract much attention as potential biomarker because tumor cells have been shown to release EVs into circulation which mirror their cellular origin. The main objective of this study is identification and detection of Pancreatic ductal adenocarcinoma (PDAC)-specific EVs in serum from the recurrence patients and patients at early stages. We performed proteomics analysis of EVs derived from serum of patients with pancreatic cancer and healthy donors. From this result, we focused on 2 proteins and performed immunoblotting for the validation of potential biomarkers in 3 stages of PDAC serum samples. These proteins were detected exclusively in EVs of PDAC patient sera including stage II. Moreover, we analyzed a total of 33 samples from 11 PDAC patients who performed surgery at three time points; before surgery, after surgery and recurrence as an early stage model. As a result, these proteins were detected in EVs derived from preoperative samples and recurrence samples. This study using unique recurrence samples as an early stage model shows that the identified EV-associated proteins have potential as early detection makers and warrant further investigation.

Category: AMED P-CREATE **Poster Number: 7**

Three-dimensional analysis of pancreatic fat by fat-water magnetic resonance imaging provides detailed characterization of pancreatic steatosis with improved reproducibility

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Background: Since pancreatic steatosis is reported as a possible risk factor for pancreatic cancer, the development of a non-invasive method to quantify pancreatic steatosis is needed. Proton density fat fraction (PDFF) measurement is a magnetic resonance imaging (MRI) based method for quantitatively assessing the steatosis of a region of interest (ROI). Although it is commonly used for quantification of hepatic steatosis, pancreatic PDFF can greatly vary depending on the ROI's location because of the patchy nature of pancreatic fat accumulation. In this study, we attempted to quantify pancreatic steatosis by fat-water MRI with improved reproducibility.

Methods: Using the MRI images of 159 patients with nonalcoholic fatty liver disease, we attempted to calculate the average PDFF of whole pancreas. We set ROIs covering the entire area of the pancreas appearing in every slice and calculated the average PDFF from all the voxels included in the pancreas. We named this average value as whole-pancreatic PDFF and evaluated the reproducibility of the measured values. In addition to whole-pancreatic PDFF, we measured the average PDFF of the pancreatic head (head-PDFF) and that of the pancreatic body plus tail separately and analyzed their correlation with the clinical characteristics of the patients. Results: The mean inter-examiner coefficient of variation of the whole-pancreatic PDFF was 11.39 %. The whole-pancreatic PDFF was correlated with age ($p = 0.039$), body mass index ($p = 0.0093$) and presence/absence of diabetes ($p = 0.0055$). The serum level of low-density lipoprotein cholesterol was inversely correlated with the head-PDFF.

Conclusion: We developed a new measurement method of the pancreatic PDFF with greater reproducibility. Using this method, we characterized pancreatic steatosis in detail. This novel measurement method allows accurate estimation of the severity of pancreatic steatosis and is therefore useful for the detailed characterization of pancreatic steatosis.