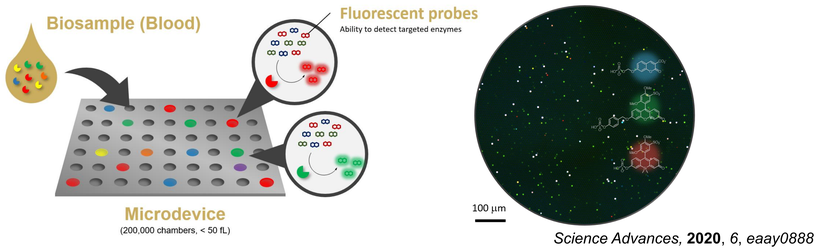
Early detection of pancreatic cancer by single-molecule enzyme activity-based liquid biopsy

Shingo Sakamoto,[1] Hideto Hiraide,[1] Junpei Hatakeyama,[1] Kazuki Takahashi,[1] Takashi Inagaki,[1] Toshihiro Uno,[1] Takashi Kobayashi,[2] Yuzo Kodama,[2] Toru Komatsu,[1,3] Yu Kagami[1]

[1]Cosomil, Inc., Tokyo, Japan; [2]Division of Gastroenterology, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, Japan; [3]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan

BACKGROUND / OBJECTIVE

- The development of liquid biopsy approaches for early detection of pancreatic ductal adenocarcinoma (PDAC) is highly desirable to broaden treatment options and improve patient survival.
- We have developed a highly sensitive enzyme activity measurement platform based on single-molecule enzyme activity-based liquid biopsy.



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- The objective of this study was to identify and evaluate single-molecule enzyme activity-based biomarkers for PDAC.

METHODS

Study design

A total of 690 plasma samples were collected: non-cancer controls (N=345), PDAC (N=108), other cancers (N=157), and pancreatic diseases (N=80) from 8 institutions in Japan and 4 biobanks in the US.

Sample selection by institution and stage distribution

Division into training and validation sets without institutional overlap

Screening

Screening was performed using more than 100 single-molecule enzyme activity assays

Criteria
- Showing PDAC-specific alteration
- Consistent across institutions
- Orthogonal to each other and expected to improve performance in combination

Identification of candidate biomarkers

Training
- Non-cancer control (N=212)
- PDAC (N=60)

Construction of the prediction model
(algorithm: random forest classifier)

Validation
- Non-cancer control (N=133)
- PDAC (N=48)
- Other cancers (N=157)
- Pancreatic diseases (N=80)

Performance evaluation

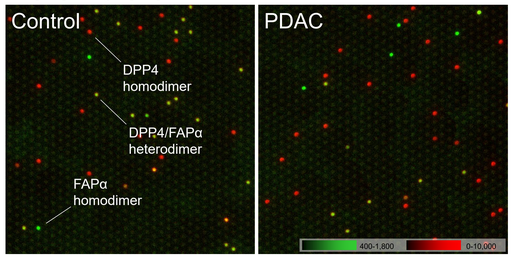
Clinical characteristics of plasma samples used in this study

		Training set		Validation set	
		PDAC (N=60)	Control (N=212)	PDAC (N=48)	Control (N=133)
Sex	Male	34	127	29	60
	Female	26	85	19	73
Age (y)	Mean [Min, Max]	67.4 [37, 86]	66.4 [46, 87]	70.0 [45, 86]	59.4 [28, 92]
Race	Asian (Japanese)	50	150	32	118
	White	10	42	12	8
	Black/African American	0	8	4	5
	Hispanic/Latino	0	12	0	2
Stage (UICC 8th ed.)	I (IA, IB)	15 (7, 8)	-	13 (8, 5)	-
	II (IIA, IIB)	10 (0, 10)	-	18 (3, 15)	-
	III	15	-	7	-
	IV	20	-	10	-
Originating institutions		– Kobe University Hospital and 5 related institutions – PrecisionMed, LLC. – REPROCELL, Inc.		– Japan Institute for Health Security – Okayama University – Dx Biosamples, LLC. – BioIVT, LLC.	

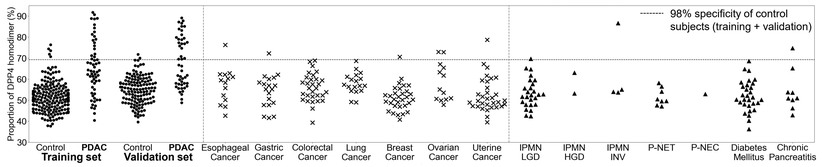
RESULTS 1: Identification of biomarkers

Four biomarkers identified in the screening

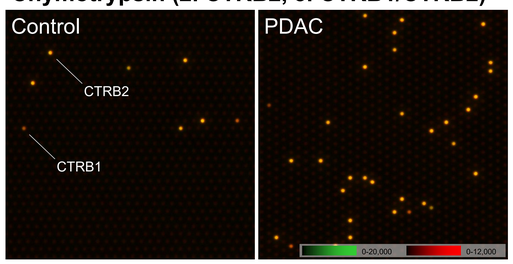
Dipeptidyl peptidase IV and Fibroblast activation protein α (1. DPP4/FAPα)



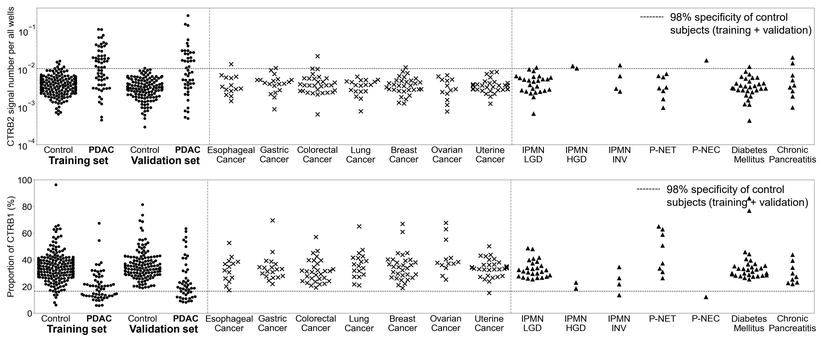
- In plasma of PDAC patients, **DPP4 homodimer / (DPP4 homodimer + DPP4/FAPα heterodimer)** was increased.
- The DPP4–FAPα heterodimer is thought to be secreted into the circulation by pancreatic α-cells or cancer-associated fibroblasts.



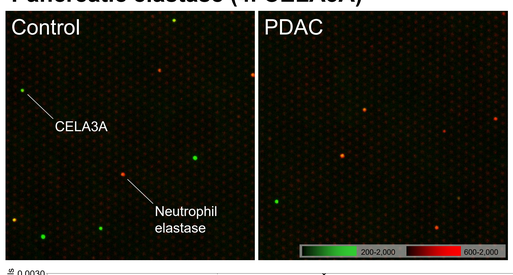
Chymotrypsin (2. CTRB2, 3. CTRB1/CTRB2)



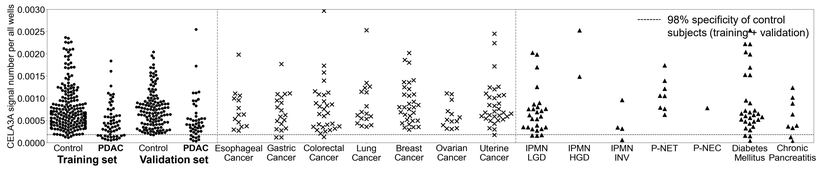
- In plasma of PDAC patients, **CTRB2** was increased and **CTRB1 / (CTRB1 + CTRB2)** was decreased.
- The results suggest that pro-chymotrypsin is present in the circulation as a result of endocrine-like pancreatic activity. Its increase in PDAC may reflect aberrant secretion of the enzyme.



Pancreatic elastase (4. CELA3A)

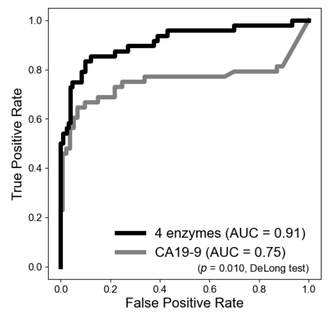


- In plasma of PDAC patients, **CELA3A** was decreased.
- Circulating elastase activity is normally suppressed by protease inhibitors such as serpins. Therefore, the detectable activity of CELA3A in plasma likely indicates their imbalance in PDAC.

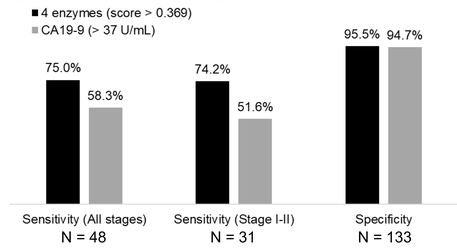


RESULTS 2: Performance evaluation (Validation set)

Performance of the combination of four biomarkers

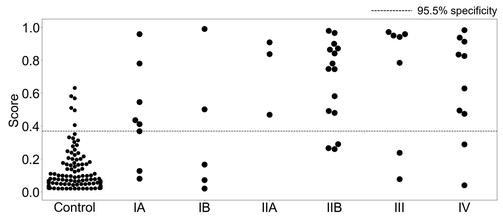


Definition of score: A probability value (0–1) calculated by the prediction model trained on the training set; higher scores indicate a higher likelihood of PDAC.



➢ The combination of four biomarkers outperformed CA19-9 in detecting PDAC compared with non-cancer controls.

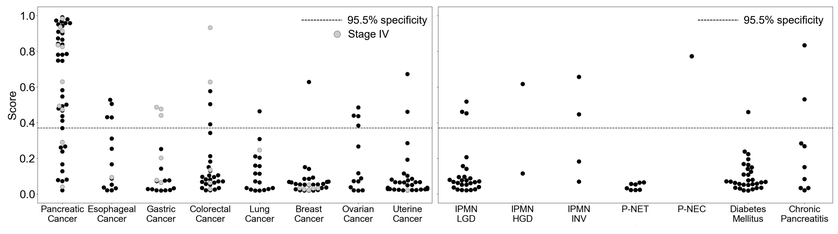
Sensitivity across PDAC stages



		Positive number (Sensitivity %)	
Stage	N	4 enzymes (score > 0.369)	CA19-9 (> 37 U/mL)
All	48	36 (75.0%)	28 (58.3%)
I-II	31	23 (74.2%)	16 (51.6%)
I	13	8 (61.5%)	5 (38.5%)
IA	8	6 (75.0%)	2 (25.0%)
IB	5	2 (40.0%)	3 (60.0%)
II	18	15 (83.3%)	11 (61.1%)
IIA	3	3 (100%)	2 (66.7%)
IIB	15	12 (80.0%)	9 (60.0%)
III	7	5 (71.4%)	4 (57.1%)
IV	10	8 (80.0%)	8 (80.0%)

➢ It also maintained high sensitivity for Stage I/II PDAC, highlighting its potential for early detection.

Positive rates in other cancers and pancreatic diseases



		Positive number (Rate %)	
	N	4 enzymes (score > 0.369)	CA19-9 (> 37 U/mL)
Esophageal cancer	14	4 (28.6%)	3 (21.4%)
Gastric cancer	19	3 (15.8%)	5 (26.3%)
Colorectal cancer	30	5 (16.7%)	1 (3.3%)
Lung cancer	18	1 (5.6%)	3 (16.7%)
Breast cancer	31	1 (3.2%)	4 (12.9%)
Ovarian cancer	13	4 (30.8%)	6 (46.2%)
Uterine cancer	32	2 (6.3%)	10 (31.3%)
Other cancers	157	20 (12.7%)	32 (20.4%)

		Positive number (Rate %)	
	N	4 enzymes (score > 0.369)	CA19-9 (> 37 U/mL)
IPMN-LGD	26	3 (11.5%)	4 (15.4%)
IPMN-HGD	2	1 (50.0%)	1 (50.0%)
IPMN-INV	4	2 (50.0%)	2 (50.0%)
P-NET	8	0 (0.0%)	0 (0.0%)
P-NEC	1	1 (100%)	0 (0.0%)
Diabetes mellitus	30	1 (3.3%)	2 (6.7%)
Chronic pancreatitis	9	2 (22.2%)	1 (11.1%)

➢ This combination further showed specificity comparable to or higher than that of CA19-9 in other cancers and pancreatic diseases.

CONCLUSIONS / PERSPECTIVES

➢ Our findings demonstrate the feasibility of single-molecule enzyme activity assay-based liquid biopsy and provide a strong foundation for future large-scale case–control and prospective studies.

ABBREVIATIONS: IPMN-LGD = intraductal papillary mucinous neoplasm with low-grade dysplasia; IPMN-HGD = IPMN with high-grade dysplasia; IPMN-INV = IPMN with an associated invasive carcinoma; P-NET = pancreatic neuroendocrine tumors; P-NEC = pancreatic neuroendocrine carcinoma.

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