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Article

Elevated polyamines in saliva of pancreatic cancer

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Abstract: Detection of pancreatic cancer (PC) at a resectable stage is still difficult because of the lack of accurate detection tests. The development of accurate biomarkers in low or non-invasive biofluids is essential to enable frequent tests, which would help increase the opportunity of PC detection in early stages. Polyamines have been reported as possible biomarkers in urine and saliva samples in various cancers. Here, we analyzed salivary metabolites, including polyamines, using capillary electrophoresis-mass spectrometry. Salivary samples were collected from patients with PC (n=39), chronic pancreatitis (CP, n=14) and controls (C, n=26). Polyamines, such as spermine, N_1-acetylspermidine, and N_1-acetylspermine, showed a significant difference between PC and C, and the combination of four metabolites including N_1-acetylspermidine showed high accuracy in discriminating PC from the other two groups. These data showed the potential of saliva as a screening test for PC.

Keywords: pancreatic cancer, saliva, metabolomics, polyamines

1. Introduction

Pancreatic cancer (PC) has the worst prognosis among all cancers and its 5-year survival rate is still under 5% [1]. The high mortality rate of PC is due to a lack of early specific symptoms and a delay in diagnosis [2]. Although recent diagnostic imaging technologies, such as computed tomography (CT), positron emission tomography-CT, magnetic resonance imaging (MRI), and endoscopic ultrasonography (EUS), have helped to improve the diagnosis of PC, and, of these, 30% of patients present with a locally advanced tumor, 50% present with metastatic disease, and only 20% are resectable at initial diagnosis [3]. Gemcitabine was approved for the treatment of advanced PC in

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2001. Gemcitabine, TS-1, multi-agent chemotherapy regimens, such as FOLFIRINOX, and molecular targeted therapeutic agent, have already showed effectiveness in the treatment of advanced PC [4]. However, these therapies only contribute to a slight extension of survival duration with slight improvement in advanced PC cases. Surgical resection remains the only potentially curative therapy. Thus, the early detection of PC is significantly important for improving its survival rate and prognosis. Currently, tumor markers including serum pancreatic enzymes and carbohydrate antigens such as CA19-9, CEA, DUPAN2 and SPAN1, are used for complementary diagnosis [5-7]. However, despite these markers, increase at the advanced stage of PC, as well as false negatives are also found in many cases. These tumor markers are not useful for early diagnosis at the time when surgery is possible. Therefore, it is important to establish a technology to detect early stage PC efficiently with low invasiveness, that is simple and inexpensive.

PC is induced by abnormalities of P53, KRAS, SMAD4 and other genes, which accelerate polyamine synthesis and consequently affect various primary pathways [8]. The effect of metabolic changes of PC on blood has been investigated to explore metabolite-based novel biomarkers to detect patients with PC [9-14]. The meta-analysis of these blood metabolomics for PC has also been reported [15]. We also recently reported the diagnostic ability of serum metabolomics [16], indicating the spread of metabolomic change from PC cells to metabolites in blood vessels as well as a range of biofluids.

Various omics technologies revealed the PC detection ability of salivary compounds and microbiomes [17-19]. We previously observed the change of metabolite concentration in salivary samples collected from PC patients [20]. Among metabolites, polyamines in non-invasively available biofluids have been reported as possible biomarkers of various cancers [21-23]. Elevation of urinary polyamines and the positive correlation between their concentrations in PC tissue is well known [24]. Salivary polyamines showed potential detection ability in breast cancer [25, 26]. However, the potential of PC detection using salivary polyamines has not been investigated.

The purpose of this study is to evaluate the potential ability of salivary polyamines to detect PCs. We utilized capillary electrophoresis-mass spectrometry (CE-MS) to quantify these metabolites and accessed their sensitivity and specificity by comparison of polyamine profiles for PC, and chronic pancreatitis (CP), and controls (C).

2. Results

Patient information is summarized in Table 1. Metabolomic analysis successfully identified and quantified 292 metabolites in saliva samples and, of these, those 142 metabolites frequently detected (at least >50% per group) were used for subsequent analyses. Score plots of PCA (Figure 1a) showed the overall metabolite concentration pattern among all samples. The score plots of C and CP aggregated while several PC plots were scattered, indicating that the metabolomic profiles of PC showed large differences compared to C and CP.

To access the discrimination ability of the salivary metabolites, the receiver operating characteristic (ROC) curve of this MLR model was developed (Figure 1b). In total, 24 metabolites showed significant differences (corrected p-value < 0.05; Mann-Whitney test) and F.C. > 4.0 between PC and (C + CP), and the 4 metabolites were selected by stepwise feature selection (Table 2). The model included alanine, N_1-acetylspermidine, 2-oxobutyrate, and 2-hydroxybutyrate. The area under the ROC curve (AUC) of this model was 0.887 (95% confidence interval [CI]; 0.784 – 0.944). As pruning, the metabolites showing the largest p-value in the model were eliminated one by one. The model with fewer parameters showed less AUC values;

The metabolites showing significant differences between PC and (C+CP) (corrected $p < 0.001$; Mann-Whitney test) included 3 polyamines (spermine, N_1-acetylspermidine, and N_1-acetylspermine) and 2-aminobutanoate (2AB). Their stage-specific concentrations are depicted in Figure 2. By multiple comparison (Steel-Dwass test), comparison between C and PC with stage III and stage IVb showed significant differences. Spermine showed significant differences in various comparisons, e.g. CP and PC in stage IVb.

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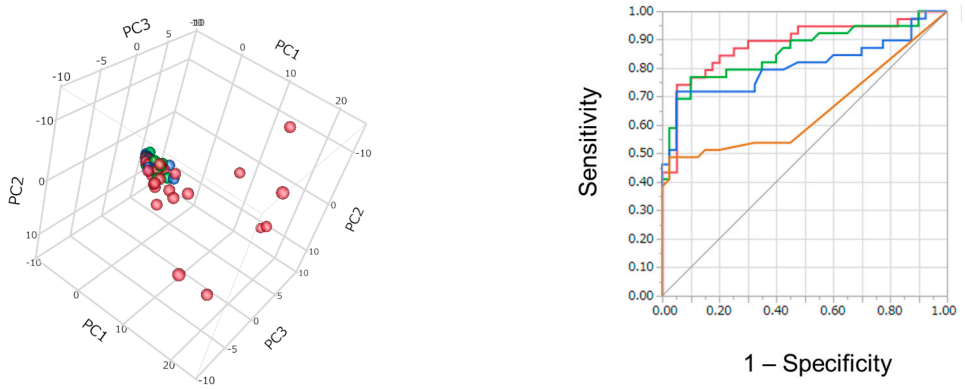
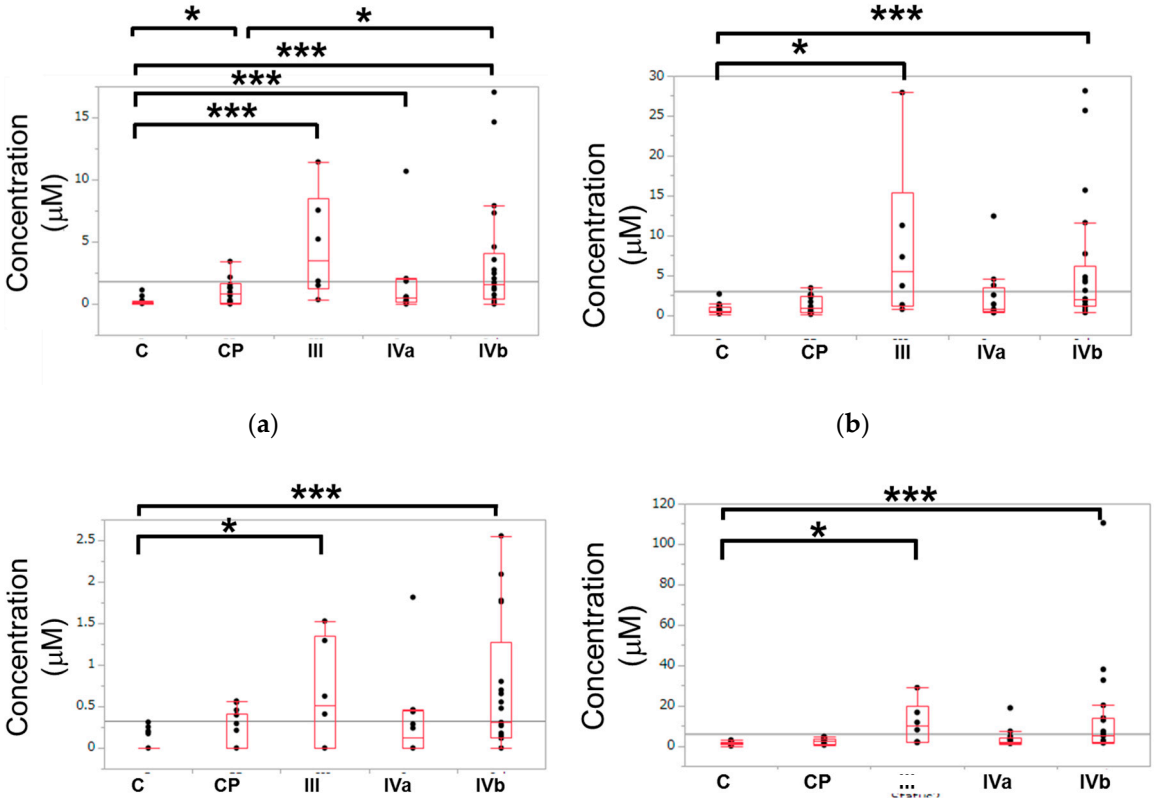


(a) (b)

Figure 1. Discrimination ability of metabolomics profile: (**a**) Score plots of PCA. Contribution ratio to first, second, and third PC (PC1, PC2, and PC3) were 34.0, 5.7, and 4.7, respectively. Blue, green, and red plots indicated C, CP, and PC, respectively; (**b**) ROC curves of MLR: red, green, blue, and orange curves indicated the MLR model with 4, 3, 2, and 1 metabolite(s), respectively, and their AUC values were 0.887 (95% CI; 0.784 – 0.944, $p < 0.0001$), 0.859 (95% CI; 0.749 – 0.925, $p < 0.0001$), 0.807 (95% CI; 0.749 – 0.925, $p < 0.0001$), and 0.653 (95% CI; 0.526 – 0.761, $p < 0.0122$), respectively. The differences of AUC of the model with 4 parameters were 0.0501, 0.0280, and <0.0001 for those with 3, 2, and 1 parameters, respectively.



(a) (b)

(c) (d)

Figure 2. Metabolite concentration: (**a**) spermine; (**b**) N_1-acetylspermidine; (**c**) N_1-acetylspermine; and (**d**) 2-aminobutanoate (2AB). *** p <0.001 and *p < 0.05 by the Steel-Dwass test.

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Table 1. Patient characteristics

Parameters	C	CP	PC	*p*-value	
n	26	14	39	-	
Age	50.8 ± 16.4	51.1 ± 12.4	66.1 ± 9.86	<0.0001	***
Sex (F/M)	13 / 13	3 / 11	18 / 21	0.189	

Table 2. MLR model

Parameters	Odds	95% CI		Parameter	95% CI		*p*-Value	
Alanine	0.990	0.980	1.00	-0.0103	-0.0203	-0.0003	0.043	*
N_1-Acetylspermidine	2.92	1.35	6.31	1.07	0.30	1.84	0.0065	***
2-Oxobutyrate	1.15	1.02	1.29	0.14	0.02	0.25	0.019	*
2-Hydroxybutyrate	1.46	1.07	1.99	0.38	0.07	0.69	0.017	*
(Intercept)	-	-	-	-2.21	-3.21	-1.21	<.0001	***

Table 3. Tumor markers and MLR

Marker	CP	PC			*p*-Value	
	(n = 14)	III (n = 6)	IVa (n =12)	IVb (n=21)		
CEA	3.46 ± 3.27	2.75 ± 1.46	7.25 ± 4.18	43.8 ± 101	0.0196	*
> 5.0ng/ml (%)	3 (21.4)	0 (0.0)	9 (75.0)	10 (47.6)		
#MV	0	0	0	0		
CA19-9	19.9 ± 21.3	2.90×10^2 ± 6.26×10^2	7.43×10^2 ± 9.98×10^2	6.25×10^3 ± 1.77×10^4	0.0016	***
> 37U/ml (%)	2 (14.3)	3 (50.0)	12 (100.0)	16 (76.2)		
#MV	0	0	0	0		
DUPAN2	74.0 ± 86.7	6.17×10^2 ± 7.67×10^2	5.28×10^2 ± 6.52×10^2	9.98×10^2 ± 6.52×10^2	0.0008	***
> 150U/ml (%)	2 (16.7)	4 (66.7)	6 (50.0)	18 (90.0)		
#MV	2	0	0	1		
SPAN1	16.2 ± 17.1	89.0 ± 1.58×10^2	3.63×10^2 ± 4.83×10^2	3.25×10^3 ± 8.44×10^3	<0.0001	***
> 30U/ml (%)	2 (18.2)	3 (50.0)	10 (83.3)	19 (95.0)		
#MV	3	0	0	1		
MLR	0.334 ± 0.266	0.800 ± 0.299	0.633 ± 0.363	0.786 ± 0.268	0.002	***
> 0.5533 (%)	2 (14.3)	5 (83.3)	7 (58.3)	16 (76.2)		
#MV	0	0	0	0		

Note: # MV indicates the number of missing values. (%) indicates the percentage of positive subjects.

Tumor markers, including CEA, CA19-9, DUPAN2, and SPAN1 of CP and PC, are summarized in Table 3. Based on the ROC curve of MLR (Figure 1b), the optimized cut-off value was calculated, and true positive ratios were also summarized in Table 3. As a computational validation test, k-fold CV was conducted using k=5, 10, and 20 with 200 random values for each. The median AUC values were 0.847 (95% CI; 0.840 - 0.846), 0.850 (95% CI; 0.847 - 0.851), and 0.852 (95% CI; 0.850 - 0.853) for k=5, 10, and 20, respectively. The resampling tests were also conducted using 200 random values, which yielded median AUC = 0.894 (95% CI; 0.889 - 0.901).

3. Discussion

We conducted CE-MS-based metabolomics for comprehensive analyses of hydrophilic metabolites, including polyamines, in saliva samples from patients with C, CP, and PC. The overall metabolite concentration patterns showed several salivary samples collected from patients with PC showing a large difference among all samples (Figure 1a).

The MLR model developed here included 4 metabolites and yielded an AUC = 0.887 (Figure 1b). As a pruning test, the AUC values between the model with 4 and 3 metabolites showed no significant difference (p = 0.0501, Figure 1b) while the model with 2 and 1 metabolites showed significantly decreased accuracy, indicating the combination of multiple metabolites contributed to enhance both sensitivity and specificity in discriminating PC patients from the other groups. Alanine was included in this model (Table 2), while no specificity for pancreatic cancer was expected because of various reports on this metabolite [27, 28]. Since not only these 4 metabolites, but also 24 metabolites showed potential as markers to discriminate PC from the others (corrected $p < 0.05$; Mann-Whitney test and F.C. > 4.0 between PC and (C + CP)), the specificity of these metabolites should be validated using larger cohorts and parameter selection.

Our computational test using k-fold CV and resample tests showed high generalization ability, since no median AUC showed distinct decrease compared to the original AUC calculated using all datasets. In particular, the differences between the upper margin of the lower limit of the 95% CI of AUC values of all validation tests were small; 0.006, 0.004, 0.003, and 0.012 for 5, 10, and 20-fold CV and resampling. Both tests indicated the high generalization ability of the model.

Among the metabolites showing high discrimination ability, 3 polyamines were included (Figure 2). Spermine showed a unique pattern, e.g. all PC groups at all stages showed significant differences compared with C and PC in stage IVb (Figure 2a). However, this metabolite also showed a significant difference between C and CP. Two acetylated-polyamines, including N_1-acetylspermidine and N_1-acetylspermine, were elevated in PC compared with stages III and IVb (Figures 2b, c). Concentrations of polyamines are tightly controlled in normal cells, e.g. ornithine by ornithine decarboxylase (ODC) [EC 4.1.1.17] which converts ornithine to putrescine, a first metabolite of the polyamine pathway, is negatively regulated by adenomatous polyposis coli (APC) tumor-suppressor gene while mutated or deleted APC would activate ODC, contributing to enhance putrescine, and subsequent metabolites, such as spermidine and spermine [8]. The acetylation of these metabolites is also activated in spermidine/spermine N_1-acetyltransferase (SSAT) in cancer cells [8]. In particular, N_1,N_{12}-diacetylspermine is known to be secreted by tumors and its concentration is elevated in urine in various cancer patients [29]. However, this metabolite was not independently detected in our CE-MS measurement conditions, i.e. adduct or fragment ion of other metabolites were detected at the same migration time. The elevation of salivary N_1-acetylspermidine and N_1-acetylspermine is considered as reasonable and the lack of a significant difference between C and PC with stage IVb might be attributed to the low number of patients.

There are several limitations that need to be acknowledged. Firstly, the number contained in the cohort of this study was quite small and validation with a larger cohort is necessary. The difference of age is also a problem of this study. In this study, we recruited only advanced PC in stage III, IVa, and IVb. The validation of PC at an early stage is the most important to access the value of the saliva-based PC screening test demonstrated here. The specificity of the elevated salivary markers also should be analyzed using different types of diseases, since polyamines in saliva samples collected from breast cancer patients were previously reported [25,26]. Our data also revealed large overlap of elevated salivary metabolites among various cancers [20]. Environmental factors also affect the salivary metabolite profiles [28] and standards of protocols to handle the saliva should also be established based on observed marker metabolites, since several salivary metabolites were unstable after the saliva collection [30]. Taken together, more rigorous validation methods are still necessary to fully evaluate the potential of salivary-based PC detection.

4. Materials and Methods

Patient Selection and Serum Collection

Sample collection was conducted at Tokyo Medical University Hospital. All patients had pancreatic cancers diagnosed histologically. All patients were recently diagnosed with primary disease and none had received any prior treatment in the form of chemotherapy, radiotherapy, surgery, or alternative therapy. No subjects had a history of prior malignancy. This study was approved by the ethics committee of Tokyo Medical University (approval no. 1560). Written informed consent was obtained from all patients and from volunteers who agreed to serve as saliva donors. Our study was carried out in accordance with the Helsinki Declaration.

Protocols for saliva collection

The saliva providers were not allowed to take any food except water intake after 21:00 on the previous day. The subjects were required to brush their teeth without toothpaste on the day of saliva collection, and had to refrain from drinking water, smoking, tooth-brushing and intense exercise from one hour before saliva collection. They were required to gargle with water just before saliva collection. Approximately 400 µl of unstimulated saliva was collected in a 50 cc polypropylene tube. A polypropylene straw 1.1 cm in diameter was used to assist the saliva collection

Sample Preparation

Saliva samples were stored at -80 ˚C until metabolomic analyses. The protocol of salivary preparation for metabolomic analyses is described elsewhere (31). All samples were collected at 08:00 am – 11:00 am. Eating and drinking were not permitted for at least 1.5 hours prior to saliva collection. Each subject rinsed their mouth with water, and their saliva was collected in a 50 ml Falcon tube on ice. Approximately 200 – 400 µl unstimulated whole saliva was collected over 5–10 min. After collection, the saliva samples were immediately stored at −80 °C until metabolite measurements.

Measurement Conditions and Processing of Raw Data

The metabolite standards, capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) instrumentation, and measurement conditions for cationic and anionic metabolites were described previously [20,31-33]. Briefly, CE-TOFMS analysis was performed using an Agilent 7100 CE system (Agilent Technologies, Waldbronn, Germany), an Agilent 6224 liquid chromatography (LC)/MS TOF system, an Agilent 1260 series isocratic HPLC pump, a G1603A Agilent CE-MS adapter kit, and a G1607A Agilent CE-ESI-MS sprayer kit (Agilent Technologies, Santa Clara, CA). For system control and data acquisition, Agilent Chemstation software was used for CE and Agilent MassHunter software was used for TOFMS.

Cationic metabolites were separated on a fused-silica capillary column (50-µm inner diameter×100-cm total length) filled with 1 M formic acid as the electrolyte. The sample solution was injected at 5 kPa for 3 s (approximately 3 nL), and a positive voltage of 30 kV was applied. The sheath liquid, methanol/water (50% v/v) containing 0.1 µM hexakis(2,2-difluorothoxy) phosphazen, was delivered at 10 µL/min. Anionic metabolites were separated using a commercially available COSMO(+) capillary column coated with a cationic polymer (Nacalai Tesque, Kyoto, Japan). Ammonium acetate solution (50 mM, pH 8.5) was used as the electrolyte. The sample solution was injected at 5 kPa for 30 s (approximately 30 nL) and a voltage of 30 kV was applied. The sheath liquid, ammonium acetate (5 mM) in methanol/water (50% v/v) containing 0.01 µM hexakis(2,2-difluorothoxy) phosphazen, was delivered at a rate of 10 µL/min. Results were automatically recalibrated relative to the masses of two reference standards in each mode. Cationic analysis used the 13C isotopic ion of a protonated methanol dimer $(2MeOH + H)^+$, *m/z* 66.06306, and protonated hexakis(2,2-difluorothoxy) phosphazen $(M + H)^+$, *m/z* 622.02896, whereas anionic analysis used the 13C isotopic ion of a deprotonated acetic acid dimer $(2CH_3COOH + H)^+$, *m/z* 120.03834, and hexakis(2,2-difluorothoxy) phosphazen + deprotonated acetic acid $(M + CH_3COOH + H)^+$, *m/z* 680.03554. Mass spectra were acquired at a rate of 1.5 cycles/sec from *m/z* 50 to 1000.

The analysis of raw data was conducted by following the typical data processing flow [27], including noise-filtering, baseline-correction, peak integration of each sliced electropherogram, estimation of accurate *m*/*z*, alignment across multiple datasets, and identification by matching the *m*/*z* value and the corrected migration time to corresponding entries in a standard library, using MasterHands (Keio University, Tsuruoka, Japan) [20]. Metabolite concentrations were calculated based on a ratio of relative area (the area divided by the area of the internal standards) between sample and standard compound mixtures.

Statistical Analysis

The Mann-Whitney test was used to access the difference of metabolite concentrations between 2 groups. The false discovery rate (Benjamini and Hochberg methods) [34] was used to correct P-values, considering multiple independent tests. Clinical values, except for continuous values, were accessed by the χ^2 test. Overall metabolomic concentrations were accessed by principal component analysis (PCA). To eliminate noise-like peaks, only frequently detected metabolites (50% of subjects of at least one group) were used for PCA. To evaluate the discrimination ability of multiple metabolites, multiple logistic regression (MLR) was conducted. Of the metabolites used for PCA, metabolites showing both significant differences (corrected p-value < 0.05 by Mann-Whitney test) and fold change (F.C.) > 4.0 of the averaged concentrations between PC and (C + CP) groups were selected. Subsequently, stepwise feature selection with backward (p > 0.05) and forward selection (p < 0.05) to eliminate multicollinearity, and an MLR model was developed. The Steel-Dwass test was used for stage-specific differences.

To access the generalization ability of MLR, two computational validations were conducted; (1) k-fold cross validation (CV). Data were randomly split into two (k: k-1) datasets and the former was used for training, the remaining data were used for validation. This was repeated k times and the prediction ability using validation datasets was used. (2) Resampling. To eliminate optimistic prediction, subjects were randomly selected, allowing redundant selection to generate the datasets with a number of subjects identical to the original datasets, the MLR model was developed and the accuracy was accessed.

The analyses were conducted using R (ver. 3.4.3) [35], JMP (ver. 13.2.0, SAS Institute Inc., Cary NC), and WEKA (ver. 3.6.13) [36].

5. Conclusions

In this study, we evaluated the discrimination ability of salivary metabolite patterns for PC. Polyamines, especially spermine, showed unique concentration patterns and a significant difference between C and PC. The combination of four metabolites showed high accuracy in discriminating PC from CP and C, and computational validation tests confirmed the high generalization ability of the developed model. Although there are several limitations, e.g. a small cohort, with only advanced PC, and no other cancer patients, the salivary metabolites including polyamines showed potential ability for screening of PC.

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Author Contributions: Takao Itoi, Motohide Shimazu, Tomoyoshi Soga, Masaru Tomita and Makoto Sunamura conceived and designed the experiments; Masahiro Sugimoto analyzed the data; Yasutsugu Asai, Atsushi Sofuni, Takayoshi Tsuchiya, Reina Tanaka, Ryosuke Tonozuka, Mitsuyoshi Honjyo, Shuntaro Mukai, Mitsuru Fujita, Kenjiro Yamamoto, Yukitoshi Matsunami, Takashi Kurosawa, Yuichi Nagakawa and Sigeyuki Kawachi collected patient samples; Miku Kaneko and Sana Ota measured metabolites; Yasutsugu Asai and Masahiro Sugimoto wrote the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Jemal, A.; Siegel, R.; Ward, E.; Hao, Y.; Xu, J.; Murray, T.; Thun, M.J. Cancer statistics, 2008. CA Cancer J Clin 2008, 58, 71-96.
2. Rosty, C.; Goggins, M. Early detection of pancreatic carcinoma. Hematology/oncology Clinics of North America 2002, 16, 37-52.
3. Canto, M.I.; Hruban, R.H.; Fishman, E.K.; Kamel, I.R.; Schulick, R.; Zhang, Z.; Topazian, M.; Takahashi, N.; Fletcher, J.; Petersen, G., et al. Frequent detection of pancreatic lesions in asymptomatic high-risk individuals. Gastroenterology 2012, 142, 796-804; quiz e714-795.
4. Papadatos-Pastos, D.; Thillai, K.; Rabbie, R.; Ross, P.; Sarker, D. Folfirinox - a new paradigm in the treatment of pancreatic cancer. Expert Rev Anticancer Ther 2014, 14, 1115-1125.
5. Kiriyama, S.; Hayakawa, T.; Kondo, T.; Shibata, T.; Kitagawa, M.; Ono, H.; Sakai, Y. Usefulness of a new tumor marker, span-1, for the diagnosis of pancreatic cancer. Cancer 1990, 65, 1557-1561.
6. Luo, G.; Liu, C.; Guo, M.; Cheng, H.; Lu, Y.; Jin, K.; Liu, L.; Long, J.; Xu, J.; Lu, R., et al. Potential biomarkers in lewis negative patients with pancreatic cancer. Annals of Surgery 2017, 265, 800-805.
7. Tsutsumi, K.; Kawamoto, H.; Hirao, K.; Sakakihara, I.; Yamamoto, N.; Noma, Y.; Fujii, M.; Kato, H.; Ogawa, T.; Ishida, E., et al. Monitoring of CA19-9 and SPAN-1 can facilitate the earlier confirmation of progressing pancreatic cancer during chemotherapy. Pancreatology 2012, 12, 409-416.
8. Gerner, E.W.; Meyskens, F.L., Jr. Polyamines and cancer: Old molecules, new understanding. Nat Rev Cancer 2004, 4, 781-792.
9. Costello, E. A metabolomics-based biomarker signature discriminates pancreatic cancer from chronic pancreatitis. Gut 2018, 67, 2-3.
10. He, X.; Zhong, J.; Wang, S.; Zhou, Y.; Wang, L.; Zhang, Y.; Yuan, Y. Serum metabolomics differentiating pancreatic cancer from new-onset diabetes. Oncotarget 2017, 8, 29116-29124.
11. Lindahl, A.; Heuchel, R.; Forshed, J.; Lehtio, J.; Lohr, M.; Nordstrom, A. Discrimination of pancreatic cancer and pancreatitis by LC-MS metabolomics. Metabolomics 2017, 13, 61.
12. Mayerle, J.; Kalthoff, H.; Reszka, R.; Kamlage, B.; Peter, E.; Schniewind, B.; Gonzalez Maldonado, S.; Pilarsky, C.; Heidecke, C.D.; Schatz, P., et al. Metabolic biomarker signature to differentiate pancreatic ductal adenocarcinoma from chronic pancreatitis. Gut 2018, 67, 128-137.
13. Tumas, J.; Kvederaviciute, K.; Petrulionis, M.; Kurlinkus, B.; Rimkus, A.; Sakalauskaite, G.; Cicenas, J.; Sileikis, A. Metabolomics in pancreatic cancer biomarkers research. Med Oncol 2016, 33, 133.
14. Wen, S.; Zhan, B.; Feng, J.; Hu, W.; Lin, X.; Bai, J.; Huang, H. Non-invasively predicting differentiation of pancreatic cancer through comparative serum metabonomic profiling. BMC cancer 2017, 17, 708.
15. Mehta, K.Y.; Wu, H.J.; Menon, S.S.; Fallah, Y.; Zhong, X.; Rizk, N.; Unger, K.; Mapstone, M.; Fiandaca, M.S.; Federoff, H.J., et al. Metabolomic biomarkers of pancreatic cancer: A meta-analysis study. Oncotarget 2017, 8, 68899-68915.
16. Itoi, T.; Sugimoto, M.; Umeda, J.; Sofuni, A.; Tsuchiya, T.; Tsuji, S.; Tanaka, R.; Tonozuka, R.; Honjo, M.; Moriyasu, F., et al. Serum metabolomic profiles for human pancreatic cancer discrimination. Int J Mol Sci 2017, 18.
17. Zhou, B.; Xu, J.W.; Cheng, Y.G.; Gao, J.Y.; Hu, S.Y.; Wang, L.; Zhan, H.X. Early detection of pancreatic cancer: Where are we now and where are we going? Int J Cancer 2017, 141, 231-241.
18. Herreros-Villanueva, M.; Bujanda, L. Non-invasive biomarkers in pancreatic cancer diagnosis: What we need versus what we have. Ann Transl Med 2016, 4, 134.
19. Torres, P.J.; Fletcher, E.M.; Gibbons, S.M.; Bouvet, M.; Doran, K.S.; Kelley, S.T. Characterization of the salivary microbiome in patients with pancreatic cancer. PeerJ 2015, 3, e1373.
20. Sugimoto, M.; Wong, D.T.; Hirayama, A.; Soga, T.; Tomita, M. Capillary electrophoresis mass spectrometry-based saliva metabolomics identified oral, breast and pancreatic cancer-specific profiles. Metabolomics 2010, 6, 78-95.
21. Niemi, R.J.; Roine, A.N.; Hakkinen, M.R.; Kumpulainen, P.S.; Keinanen, T.A.; Vepsalainen, J.J.; Lehtimaki, T.; Oksala, N.K.; Maenpaa, J.U. Urinary polyamines as biomarkers for ovarian cancer. Int J Gynecol Cancer 2017, 27, 1360-1366.
22. Tsoi, T.H.; Chan, C.F.; Chan, W.L.; Chiu, K.F.; Wong, W.T.; Ng, C.F.; Wong, K.L. Urinary polyamines: A pilot study on their roles as prostate cancer detection biomarkers. PLoS One 2016, 11, e0162217.

23. Xu, H.; Liu, R.; He, B.; Bi, C.W.; Bi, K.; Li, Q. Polyamine metabolites profiling for characterization of lung and liver cancer using an LC-tandem MS method with multiple statistical data mining strategies: Discovering potential cancer biomarkers in human plasma and urine. Molecules 2016, 21.
24. Loser, C.; Folsch, U.R.; Paprotny, C.; Creutzfeldt, W. Polyamine concentrations in pancreatic tissue, serum, and urine of patients with pancreatic cancer. Pancreas 1990, 5, 119-127.
25. Takayama, T.; Tsutsui, H.; Shimizu, I.; Toyama, T.; Yoshimoto, N.; Endo, Y.; Inoue, K.; Todoroki, K.; Min, J.Z.; Mizuno, H., et al. Diagnostic approach to breast cancer patients based on target metabolomics in saliva by liquid chromatography with tandem mass spectrometry. Clinica Chimica Acta 2016, 452, 18-26.
26. Tsutsui, H.; Mochizuki, T.; Inoue, K.; Toyama, T.; Yoshimoto, N.; Endo, Y.; Todoroki, K.; Min, J.Z.; Toyo'oka, T. High-throughput LC-MS/MS based simultaneous determination of polyamines including n-acetylated forms in human saliva and the diagnostic approach to breast cancer patients. Anal Chem 2013, 85, 11835-11842.
27. Sugimoto, M.; Kawakami, M.; Robert, M.; Soga, T.; Tomita, M. Bioinformatics tools for mass spectroscopy-based metabolomic data processing and analysis. Current Bioinformatics 2012, 7, 96-108.
28. Sugimoto, M.; Saruta, J.; Matsuki, C.; To, M.; Onuma, H.; Kaneko, M.; Soga, T.; Tomita, M.; Tsukinoki, K. Physiological and environmental parameters associated with mass spectrometry-based salivary metabolomic profiles. Metabolomics 2013, 9, 454-463.
29. Soda, K. The mechanisms by which polyamines accelerate tumor spread. J Exp Clin Cancer Res 2011, 30, 95.
30. Wang, Q.; Gao, P.; Wang, X.; Duan, Y. Investigation and identification of potential biomarkers in human saliva for the early diagnosis of oral squamous cell carcinoma. Clinica Chimica Acta 2014, 427, 79-85.
31. Ishikawa, S.; Sugimoto, M.; Kitabatake, K.; Sugano, A.; Nakamura, M.; Kaneko, M.; Ota, S.; Hiwatari, K.; Enomoto, A.; Soga, T., et al. Identification of salivary metabolomic biomarkers for oral cancer screening. Sci Rep 2016, 6, 31520.
32. Okuma, N.; Saita, M.; Hoshi, N.; Soga, T.; Tomita, M.; Sugimoto, M.; Kimoto, K. Effect of masticatory stimulation on the quantity and quality of saliva and the salivary metabolomic profile. PLoS One 2017, 12, e0183109.
33. Ishikawa, S.; Sugimoto, M.; Kitabatake, K.; Tu, M.; Sugano, A.; Yamamori, I.; Iba, A.; Yusa, K.; Kaneko, M.; Ota, S., et al. Effect of timing of collection of salivary metabolomic biomarkers on oral cancer detection. Amino Acids 2017, 49, 761-770.
34. Storey, J.D.; Tibshirani, R. Statistical significance for genomewide studies. Proceedings of the National Academy of Sciences of the United States of America 2003, 100, 9440-9445.
35. Ihaka, R.; Gentleman, R. R: A language for data analysis and graphics. J Comput Graph Stat 1996, 5, 299-314.
36. Witten, I.H.; Frank, E.; Hall, M.A.; Pal, C.J. Data mining: Practical machine learning tools and techniques. Morgan Kaufmann: 2016.