Differences in the Electric Potential of Pancreatic Head Cancer Tissues

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Key words: Electrical Potential, Pancreatic Cancer, pH, Chemotherapy, Warburg Effect

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ABSTRACT

Identifying the electrical properties of cancer relies on our in-depth understanding of the electric potential (EP) of cancer tissues. This study aimed to investigate these EP properties in 49 pancreatic head cancer tissues using a digital multimeter. The anode was placed at the central side of the tumor; the electric potential differences (EPDs) between cancer and cancer, cancer and noncancerous, noncancerous and noncancerous lesions at approximately 1 cm intervals following resection were evaluated. Pathological evaluation identified 30 of these samples as pancreatic invasive ductal carcinoma (PIDC, 10 without preoperative chemotherapy and 20 after chemotherapy), seven other pancreatic cancers, three tumors of Vater's ampulla (VA), and eight extrahepatic cholangiocarcinomas (EHCC) samples. We also evaluated the differences in the pH data for cancer and noncancerous lesions in nine of our PIDC samples. Our data suggest that the EP of pancreatic cancer tissues is higher than that of noncancerous tissues, especially in PIDCs. We also noted that the EPD was highest when comparing cancer and noncancerous lesions. Additionally, PIDC tissues presented with low pH; the pH difference between cancer and noncancerous sites was significantly correlated with EPD ($p = 0.011$). These EPDs were also correlated with tumor size in PIDC and inversely correlated with their response to chemotherapy. The EP values for

both the cancer and noncancerous sites in both the VA tumors and EHCC samples were not significantly different, whereas EPD in PIDC correlated with tumor extension and viable tumor content, suggesting that EPD might be useful for evaluating the viability and effectiveness of neoadjuvant chemotherapy.

Key words: Electrical Potential, Pancreatic Cancer, pH, Chemotherapy, Warburg Effect

INTRODUCTION

The correlation between cancers, their electric potential (EP), and the electrical characteristics of cancer tissues has been a subject of evaluation for more than 50 years. Endo et al. were the first to report a method for diagnosing cancer infiltration using differences in the resting EP of suspect tissues [3]. In their study, they described a difference in the electric potential (EPD) between the tumor and noncancerous tissues of both patients with cervical cancer and mice with transplanted cancer, facilitating the diagnosis of cancer using EPD. Later, Woodrough et al. investigated the EPD between basal cell carcinoma of the skin, benign inflammation, and normal tissues and concluded that skin cancers may exhibit positive EP when compared to that of normal or benign tissues [11]. Marino et al. measured the EP of breast tissues in patients with permissible breast masses and reported that the EP was only significantly positive in patients with cancerous masses [5]. In 2018, Cheng and Fu investigated the effective permeability of microwaves using breast tissue and reported that breast cancer tissues exhibited substantially higher conductivity than normal breast tissues and benign tumors, confirming that EPD can be used to detect breast cancer and further supporting its potential application in cancer diagnosis [1]. Several recent studies have focused on the impedance and dielectric constant of cancer cells; however, most of these evaluations have focused on the electrical

characteristics of individual cancer cells.

Here, we investigated the EP of cancer tissues and EPD between cancer and noncancerous tissues when cancer cells gather to form large tumors such as pancreatic head cancers.

MATERIALS AND METHODS

Patients

We recruited 49 patients diagnosed with pancreatic head tumors who underwent surgery between August 2018 and April 2021 for this study. We then immediately evaluated the EPD and pH of these resected specimens in our pathological laboratory to facilitate pathological diagnosis. The pathological findings of these 49 tumors identified 30 as pancreatic invasive ductal carcinomas (PIDC), four as intraductal papillary mucinous neoplasms (IPMNs), two as neuroendocrine neoplasms (NENs), one as a solid-pseudopapillary neoplasm, eight as extrahepatic cholangiocarcinomas (EHCCs), and four as tumors of Vater's ampulla (VA) (three adenocarcinomas and one adenoma) (Table 1).

The clinical profiles of these patients are summarized in Table 1. The patients raged in age from 35 to 90 years with a mean of 69.1 and median of 69 years. A total of 29 were male and 20 female and the pathological findings of the resected specimens and their

TNM factors were determined using the 8th edition of the International Union Against Cancer and the American Joint Committee on Cancer TNM Classification of Malignant Tumors [4].

Neoadjuvant chemotherapy (NAC) was performed according to the guidelines for pancreatic cancer and included gemcitabine and S-1 combination therapy or gemcitabine, abraxane, and S-1 combination therapy.

Measurement of Electric Potential (**EP**)

We used a digital multimeter (PM3, SANWA, Tokyo, Japan) to measure the EPD between cancer and cancer, cancer and noncancerous, and noncancerous and noncancerous tissues at intervals of approximately 1 cm across the length of the surgically resected specimens brought into the pathology laboratory for preparing resected tissues to evaluate histological findings by pathologists' evaluation. The measurement interval might influence the EP, we counteracted this by making sure that both electrodes were placed in parallel, ensuring the even distribution of our 1 cm intervals. The tumor was cracked by a pathologist, and the cross-section of the tumor and its surrounding noncancerous tissue were measured using the gold-plated electrodes of the potential measuring device. These measurements were all performed with the red electrode (anode)

placed at the center of the cancer tissue and the black electrode (cathode) placed in the normal tissue (Fig. 1).

PH measurement

We used a benchtop water quality PH meter (PH HORIBA's desktop pH meter F71、HORIBA Advanced Techno, Co, Ltd, Kyoto, Japan) and ISFETPH electrode 0040N-10D (HORIBA Advanced Techno, Co, Ltd.) to compare the pH of the cancer and noncancerous tissues in 17 cases. The difference in pH was then calculated by comparing the pH data from the cancer and noncancerous portions of the resected tissues.

Validation of the measurement process

EP was measured in all tissues immediately after resection in all 49 cases, and pH values were also measured in nine PIDC samples at the same time. These 9 samples were resected after chemotherapy. All of the measurement images were stored as movies and recorded using a digital camera to capture the changes in electrode positioning and measurement might be evaluated clearly. After pathological examination of these samples, histological findings and images of the tumor specimens were compared to confirm the position of the measurement, the degree of infiltration, the rate of necrosis and exact

tumor size (Fig. 1).

Histological findings

All specimens were evaluated using hematoxylin and eosin staining by clinical pathologists and the pathological diagnoses of these tumors are described in Table 1. In the tumors resected from patients receiving NAC the rates of tumor necrosis were routinely estimated by clinical pathologists. These rates were evaluated the rates of cancer cells and mesenchymal tissues in the H-E-stained specimens and conducted into the pathological report in each case.

Statistical analysis

All statistical analyses were performed using SPSS software version 17.0. Continuous data are expressed as the mean ± standard deviation, and statistical analyses were completed using Fisher's exact test, Student's *t-test*, or Spearman's correlation, as appropriate. Association between two quantitative values was assessed using Pearson correlation or Spearman correlation when the data did not follow normal distribution. All reported *P-values* were decided using two-sided evaluations, and statistical significance was set at $p < 0.05$.

Ethical approval

Our study was approved by the Hiroshima University institutional review board (E-189). Written informed consent was obtained from all patients, and all evaluations and collections were completed in accordance with the guidelines of the Declaration of Helsinki [6].

RESULTS

The EPD for the cancer and noncancerous lesions in each case are described in Fig. 2 with the data categorized by its origin. The average EPD of PIDCs with no NAC was 56.24 ± 29.46 mV (n = 10), which was significantly higher than that of the other tumor tissues evaluated here (n = 19, 8.11 ±32.90, P = 0.0006).

We then compared the EPD values for these PIDC samples resected with no NAC as follows: cancer and cancer, cancer and noncancerous, and noncancerous and noncancerous lesions (Fig. 3). The EPDs between the cancer and noncancerous lesions were significantly higher than those of the other two groups (P = 0.015 and P =0.045, respectively), indicating that cancer tissues of PIDC might be positively charged.

We then compared the changes in the EPD across the long axis for cancerous

versus noncancerous sites (Fig. 4). These evaluations then revealed that as the length of the long axis increased so the potential difference between the cancerous and noncancerous tissues of PIDC with no NAC increased ($n = 10$, $P < 0.01$). The correlation coefficient is 0.545 ($p = 0.0093$). However, this trend was reversed in the EHCC samples where EPD decreased with increasing diameter (data not shown).

The disappearance rates of cancer cells in PIDCs resected followed by NAC were routinely evaluated by professional pathologist s ranged 10-90% ($n = 18$, mean 39.4%). Fig 5A demonstrates the relationship between these disappearance rates and the EPD between cancerous and noncancerous sites in PIDCs including those resected with no NAC. These data identified an inverse relationship between these two factors with decreasing EPD facilitated a significant increase in cancer cell disappearance rates following NAC (Fig. 5A, $\gamma = -0.524$, $P = 0.036$, $n = 28$). This was later validated in the histological evaluations which revealed that PIDC specimens with low EPDs experienced increased tumor fibrosis following NAC (Fig. 5B).

Measurement of pH at cancer and noncancerous sites

In the PIDC samples resected following NAC The pH levels of the cancer sites ($n = 9$) were shown to be between 6.73 and 7.85 (mean: 6.98), which were significantly lower than those of noncancerous sites (7.21–7.98, mean: 7.54) ($P = 0.011$). In addition,

our evaluations revealed that these differences in pH were significantly correlated with EPD in PIDC specimens (Fig. 6, P = 0.011). These data suggest that a decrease in pH may contribute to the increased EP of PIDC cancer tissues.

DISCUSSION

Cancer tissue, especially pancreatic cancer tissues, are assumed to exhibit specific potential changes that allow them to be differentiated from their noncancerous adjacent tissues. This difference is believed to be derived from the difference in the pH of the cancer and noncancer tissues, which is supported by the Nernst equation [10], which states that when the pH decreases, the potential increases. This change in pH is largely attributed to the Warburg effect, which mediates the accumulation of lactic acid in pancreatic cancer cells resulting in significant changes in the pH of these tissues [9]. The Warburg effect is a term used to describe the phenomenon in which cancer cells produce ATP via glycolysis rather than oxidative phosphorylation, even under aerobic conditions. Here, glucose does not enter the mitochondria after being metabolized via the glycolysis system, resulting in the accumulation of lactic acid [8]. Previous reports have also demonstrated that the development of pancreatic cancer is accelerated by the overexpression of endoplasmic reticulum oxidoreductase 1 alpha that promotes the Warburg effect and increases the

lactic acid concentration in tumor tissues. Here, our data show that the EPD was higher in bigger tumors, suggesting that EPD may increase with tumor growth. Conversely, EPD was inversely correlated with tumor disappearance rates in pancreatic cancer tissues resected after NAC suggesting that differences in the EP between cancer and noncancerous tissues may reflect the number of viable cancer cells remaining in the resected tissues. Histological examination supported these conclusions when it revealed that tissues with low EPD also presented with a lower percentage of viable cancer cells. Therefore, EPD may be a useful marker for evaluating the viable tumor content in pancreatic organs.

However, this effect is not observed in every type of tumor. Here, we report that tumors of the VA demonstrate low EPDs, which might be correlated with their small size and their tendency to be benign in these patients. Conversely, extrahepatic EHCC shows a decrease in EPD between cancerous and noncancerous tissues, suggesting that the EP of the cancerous tissues is decreased when compared to their healthy control. Pastore et al. reported that EHCC reverses the Warburg effect by altering its metabolism from aerobic glycolysis to oxidative phosphorylation following the upregulation of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) [2, 7]. As a result, lactic acid production is reduced in EHCC, and the potential difference between the cancer and

normal tissues is significantly reduced when compared to that of PIDC. Therefore, cancer cell EP may differ based on the origin of the cancer.

In conclusion, changes in the EP of cancerous tissues in specific types of pancreatic cancers are likely due to the Warburg effect, and EPD may be correlated with tumor progression. EPD decreases in treated tumor specimens with higher tumor disappearance rates. EPD might also be useful for the evaluation of tumor malignancy grading and the effectiveness of chemotherapy in pancreatic cancer.

In this report, we only measured at the cracked face of pancreas head cancer tissues. The EP might be different between surface and center of tumors and among the cancers derived from various tissues, In fact EP did not charge in EHCC, suggesting that EP should be independently evaluated for different types of cancers. And we should clarify the reason of the EPD in cancers and apply to use this information for applying new therapeutic approach as well as evaluating prognostic factors including the effect of chemotherapy in future.

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Author contributions:

SI, YM, and EH designed the study. YM and KU provided the clinical materials. KM and KA suggested evaluating the EPD in each of the pathological specimens and performed the histological analysis. TH, MK, and SU analyzed the EPD and pH data.

Conflicts of Interest: The authors have no conflicts of interest to declare.

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FIGURE LEGENDS

Fig. 1. Image demonstrating our experimental set up to evaluate the electric potential difference between two sites. We used a digital multimeter (PM3, SANWA, Tokyo, Japan) to measure the electric potential difference (EPD) between cancer and cancer tissues, cancer and noncancerous tissue, and noncancerous and noncancerous tissues at intervals of approximately 1 cm across the surgically resected specimens. Red arrows indicate the positive node, black arrows indicate the negative node, and blue arrows indicate the tissue types. Each case was measured in the same order.

Fig. 2. Electric potential difference (EPDs) between cancer and noncancerous tissues. Pancreatic invasive ductal carcinomas (PIDCs) prior to any neoadjuvant chemotherapy (n = 10) showed significantly higher EPDs than extrahepatic cholangiocarcinoma (EHCC) and tumors of Vater's ampulla (VA)) (P = 0.0001, and P = 0.0018, respectively) but no significantly difference from IPMN (intraductal papillary mucinous neoplasms).

IPMN: intraductal papillary mucinous neoplasms, NENs: neuroendocrine neoplasms, SPN: solid-pseudopapillary neoplasm

Fig. 3. Electric potential difference (EPDs) between different sites across resected PIDCs

with no NAC (n = 10). The graph represents the EPD values at 1 cm intervals when compared between cancer and cancer, cancer and noncancerous, and noncancerous and noncancerous sites. Our data reveal that the EPD between cancer and noncancerous lesions were significantly higher than those at other sites.

Fig. 4. Correlation between electric potential difference (EPDs) and the length of the PIDC axis. We noted a significant correlation between the EPD and the length of the long axis of size of the PIDC tumor prior to any neoadjuvant chemotherapy (NAC) (correlation coefficient $\gamma = 0.545$, $P = 0.0093$).

Fig. 5 Correlation between electric potential difference (EPDs) and tumor cell disappearance rates in pancreas invasive ductal carcinomas (PIDC) following neoadjuvant chemotherapy (NAC). A: EPD values demonstrated a significant inverse correlation with the disappearance rate of tumor cells when samples were evaluated using histological examination. The cases at 0% were resected without NAC.

(correlation coefficient $\gamma = - 0.524$, $P = 0.036$)

B. Histological evaluations of the PIDC specimens. A) 10% disappearance rate; B) 30% disappearance rate; C) 70% disappearance rate; D) 90% disappearance rate. The cancer

specimens with the highest disappearance rates also revealed the highest degree of fibroblastic substitution for the removed cancer tissues.

Fig. 6 Correlation between EPD and pH differences in pancreas invasive ductal carcinoma (PIDC). Differences in EPD and pH demonstrated a significant inverse correlation ($\gamma = -0.871$, $P = 0.011$) in PIDC tissues resected following neoadjuvant chemotherapy (NAC) ($n = 9$).

Table 1 Clinical profile of the patients

No	Cancer	Age (yrs)/ sex	major axis (mm)	INF	ly	v	ne	mpd	pT	N	M	NAC	TDR (%)
1	PIDC	83/M	38	c	1	0	1	—		1	0	ND	
2	PIDC	61/M	22	b	3	3	2	0		2	0	ND	
3	PIDC	74/F	25	c	2	2	3	0		1	0	ND	
4	PIDC	74/F	15	b	1	0	1	0		1	0	ND	
5	PIDC	78/F	40	c	1	2	3	0	2	1	0	ND	
6	PIDC	81/F	35	c	0	1	1	1	2	2	0	ND	
7	PIDC	67/M	44	c	0	0	2	0	3	1	0	ND	
8	PIDC	81/M	30	c	1	2	1	0	2	2	0	ND	
9	PIDC	69/F	24	b	1	1	2	1	2	1	0	ND	
10	PIDC	90/F	19	c	1	2	3	0	1c	1	0	ND	
11	PIDC	76/M	13	c	0	0	1	0	1c	0	0	done	90
12	PIDC	57/M	30	c	0	1	3	0	2	0	0	done	30
13	PIDC	75/M	20	b	0	1	1	-		0	0	done	70
14	PIDC	71/M	25	b	1	1	3	0	2	1	0	done	20
15	PIDC	72/M	58	c	1	1	3	0	3	2	0	done	10
16	PIDC	64/M	25	b	1	0	1	+	2	0	0	done	30
17	PIDC	54/F	20	c	1	0	1	0	1c	0	0	done	60
18	PIDC	64/M	22	b	1	1	0	(-)		2	0	done	UD
19	PIDC	47/M	53	b	2	2	2	1	3	1	0	done	20
20	PIDC	59/F	35	c	1	1	3	0	2	1	0	done	40
21	PIDC	35/F	18	c	0	1	1			1	0	done	20
22	PIDC	85/F	21	c	0	1	3	1	2	1	0	done	30
23	PIDC	67/M	32	c	1	2	3	1	2	0	0	done	40
24	PIDC	57/M	42	c	1	2	3	1	3	1	0	done	70
25	PIDC	83/F	25		0	0	1	1	2	1	0	done	UD
26	PIDC	89/M	30	c	0	1	2	1	3	0	0	done	40
27	PIDC	75/M	43	c	1	0	1	1	3	1	0	done	30
28	PIDC	73/F	30	c	1	1	1	1	2	0	0	done	30
29	PIDC	81/F	39	c	0	1	1	1	2	0	0	done	20
30	PIDC	75/M	40	c	c	0	3	1	2	2	0	done	60

31	IPMN	57/F	35	b	0	0	0	0	Tis	0	0	ND
32	IPMN	61/M	45	a	0	0	0	0	Tis	0	0	ND
33	IPMN	71/F	20	b	0	0	0	1	Tis	0	0	ND
34	IPMN	75/F	45	b	0	0	0	1		0	0	ND
35	NENs	57/M	5	a	0	0	0	0	1	0	0	ND
36	SPN	52/F	20	b	0	0	1	0		0	0	ND
37	NENs	59/M	14	a	0	0	0	0		0	0	ND
38	Ac AoV	66/M	8	b	0	0	1		1a	0	0	ND
39	Tubular adenoma of VA	55/F								0	0	ND
40	AC in adenoma of VA	67/M			0	0				0	0	ND
41	AC VA	66/M	35	b	1a	0			3a	0	0	ND
42	EHCC	65/M		c	1	2	3		2a	0		ND
43	EHCC	55/M	40	c	1	2	2		1	1		ND
44	EHCC	86/M	40	c	1	1	3		3	1		ND
45	EHCC	69/F	35	c	1	0	1		2	0		ND
46	EHCC	86/F	28	c	2	2	3		1	0		ND
47	EHCC	68/F	25	c	3	2	3		3	2	1	ND
48	EHCC	71/F	45	c	1c	1c			3	2	0	ND
49	EHCC	83/F	53	c	1a	1a			1	1	0	ND

yrs: years, M: men, F; female, NAC: neoadjuvant chemotherapy, TDR: tumor disappearance rate, PIDC: invasive ductal carcinoma of pancreas, IPMN: intraductal papillary mucinous neoplasms, NENs: neuroendocrine neoplasms, SPN: solid-pseudopapillary neoplasm, EHCC: extrahepatic cholangiocarcinoma, AC: adenocarcinoma, VA: Vater's ampulla, ND: not done, UD: undetermined

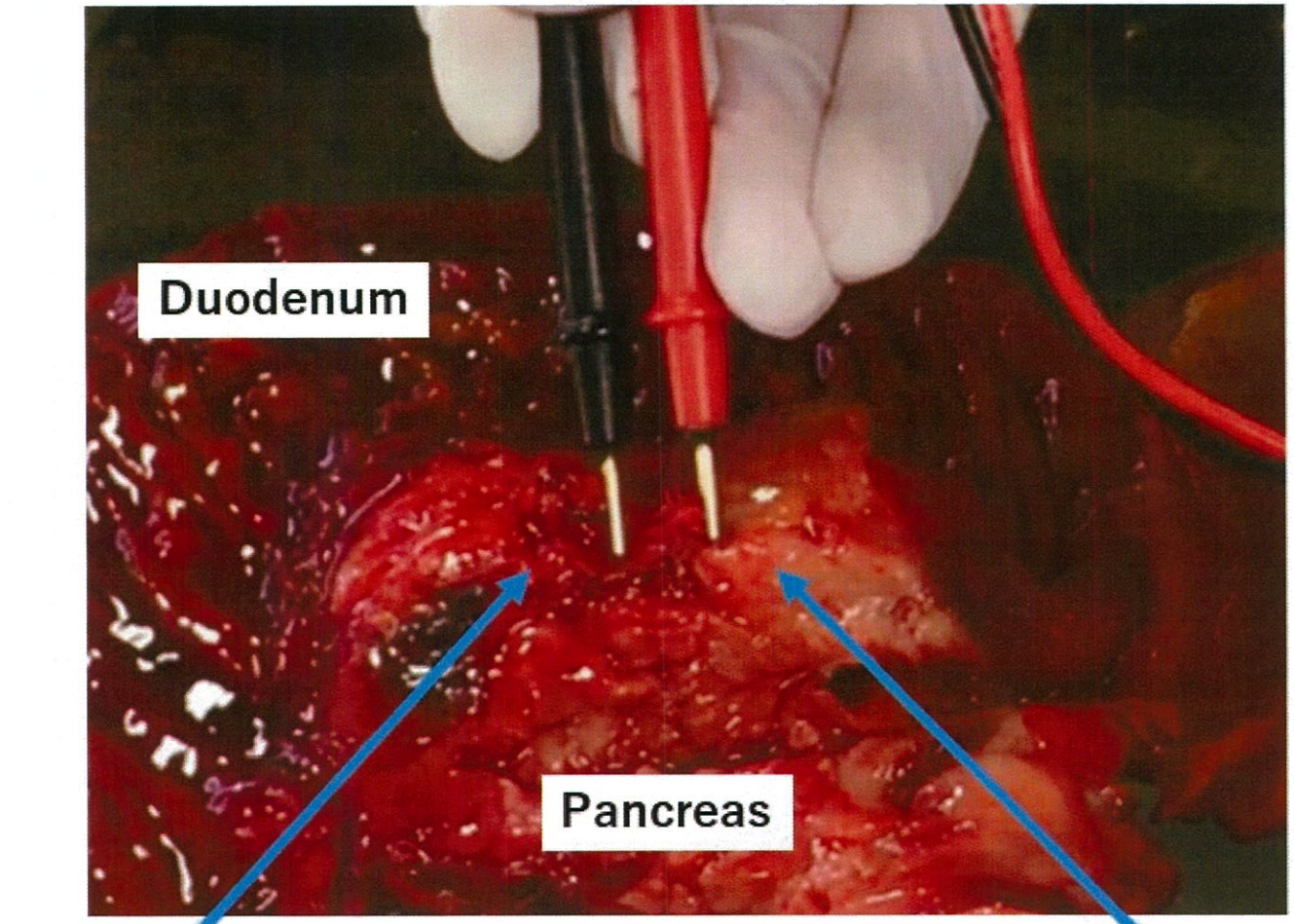

Fig. 1

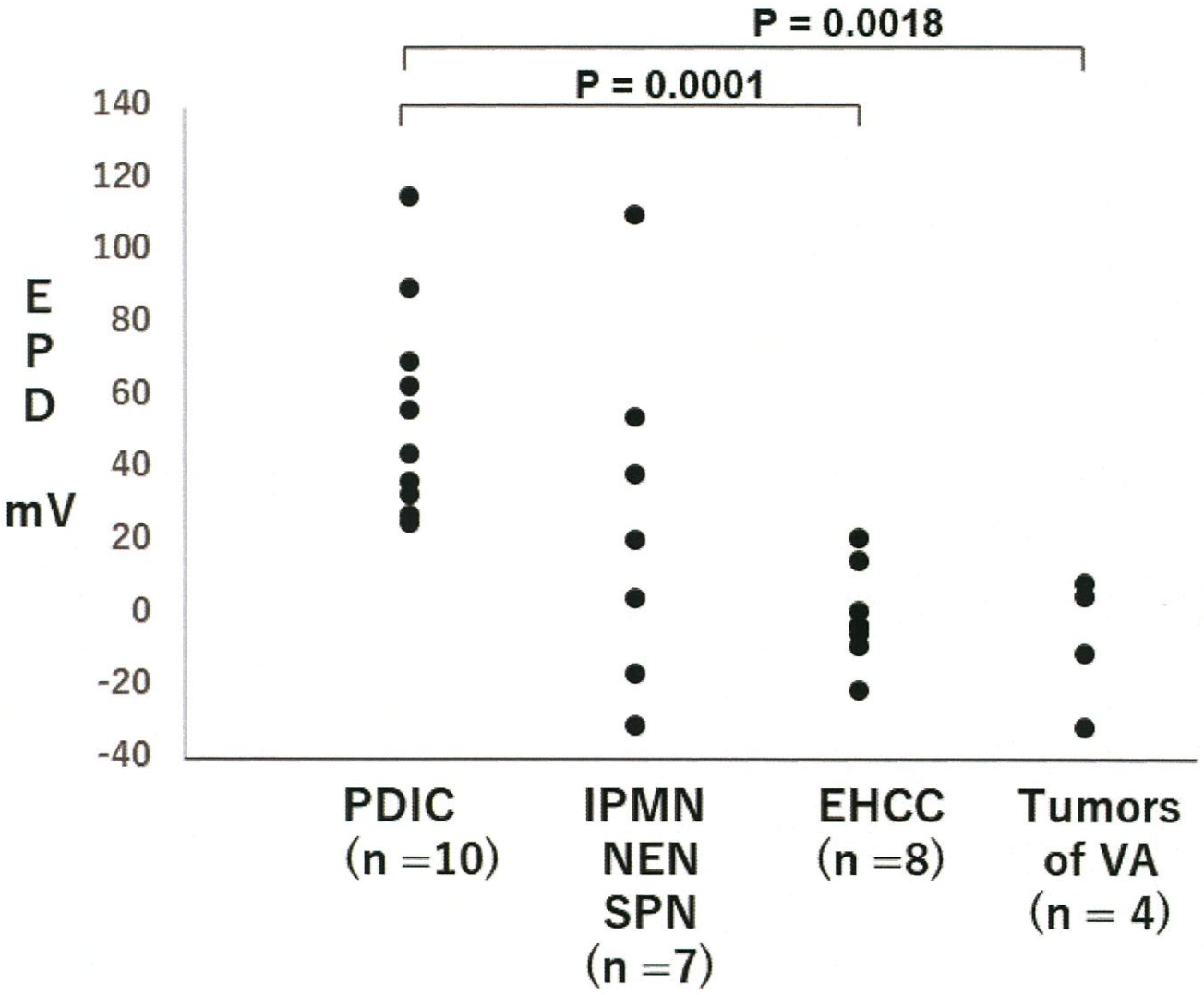

Fig. 2. Electric power difference (EPDs) between cancer and noncancerous tissues

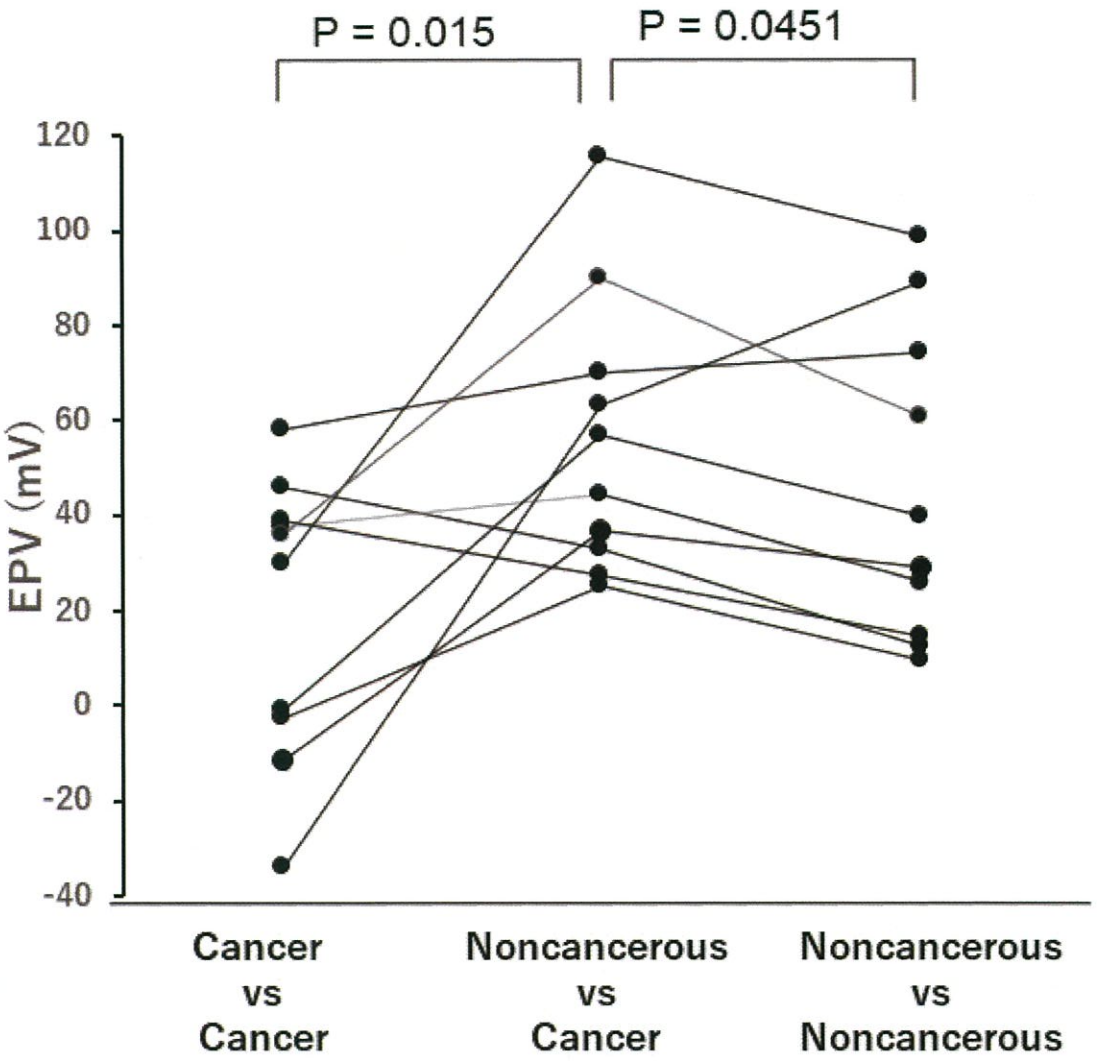

Fig. 3. Electric power difference of tissues in PIDCs

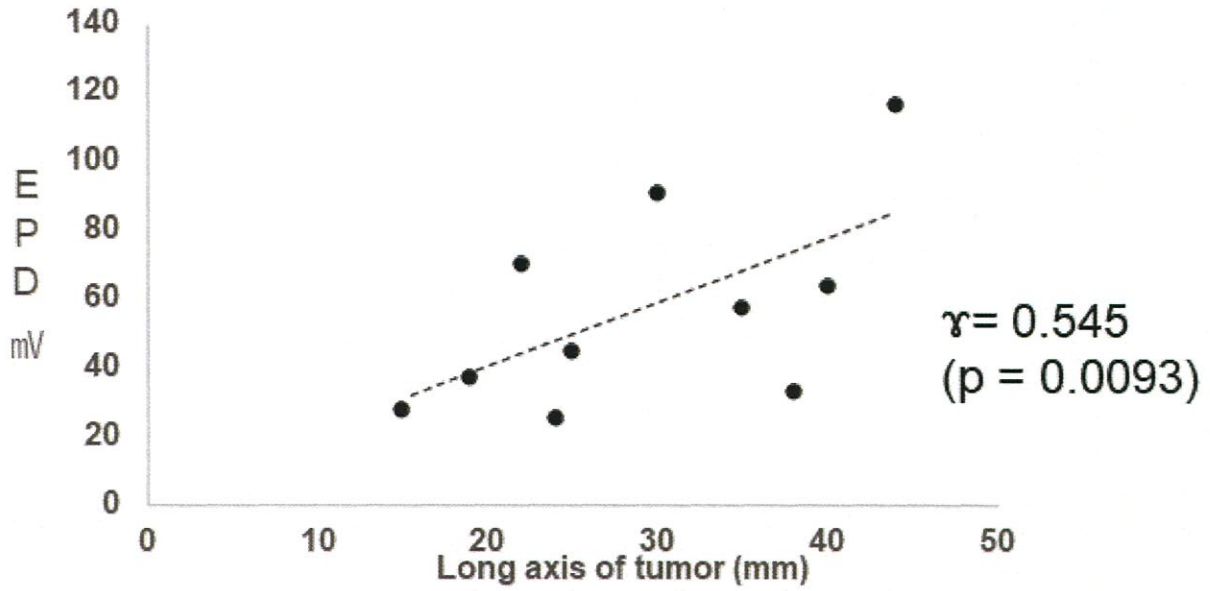

Fig. 4. Correlation between Electric power difference (EPDs) and long axis of PIDCs

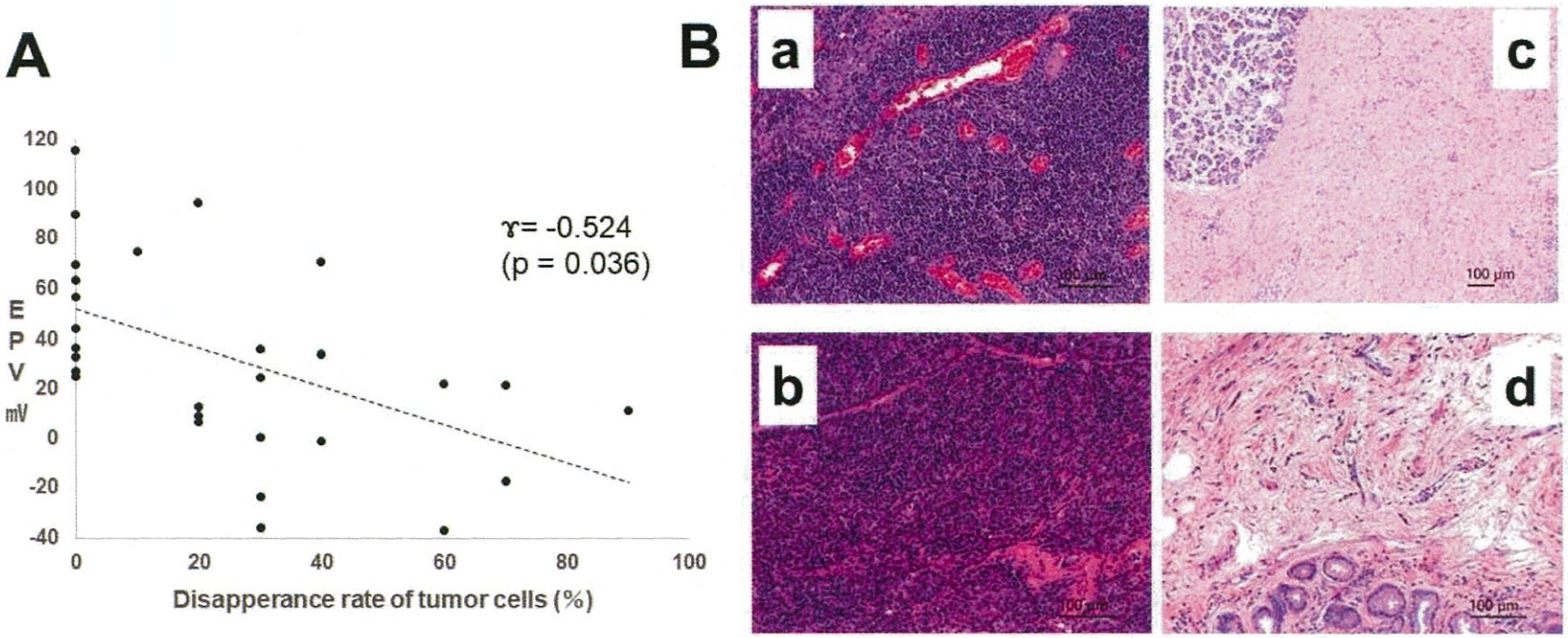

Fig 5. Correlation between electric potential difference (EPDs) and disappearance rates of tumor cells in pancreas invasive ductal carcinomas (PIDC) following neoadjuvant chemotherapy (NAC).

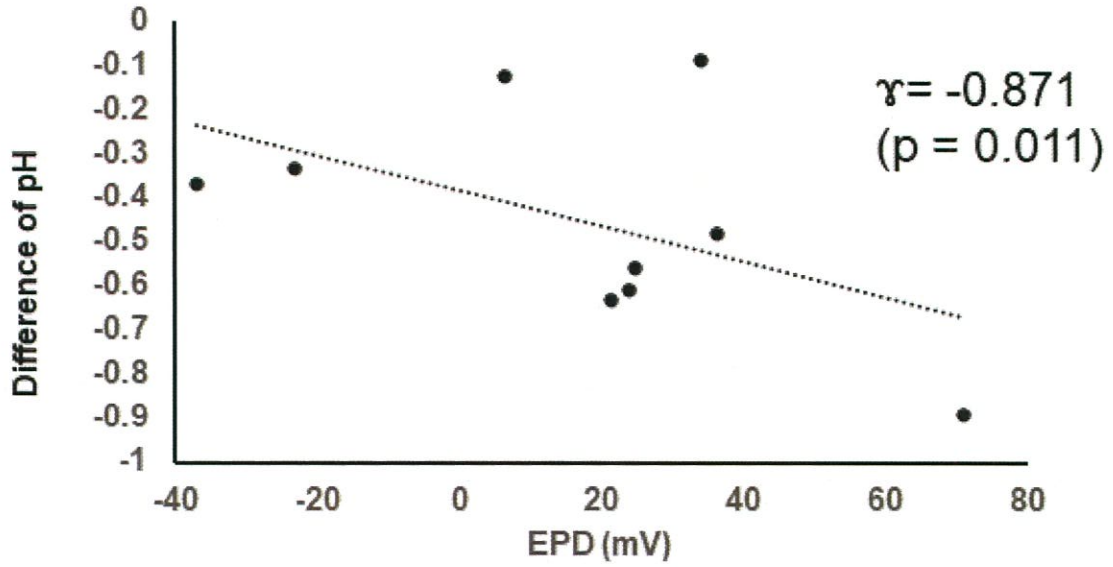

Fig 6. Correlation between EPDs and difference of pH in pancreas invasive ductal carcinoma (PIDC)