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Pancreatic ductal adenocarcinoma with acinar-to-ductal metaplasia-like cancer cells shows increased cellular proliferation

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ABSTRACT

Background/objectives: Acinar-to-ductal metaplasia (ADM) has been shown to contribute to the development of pancreatic ductal adenocarcinoma (PDAC) in genetically engineered mouse models, but little is known about whether acinar cell plasticity contributes to carcinogenesis in human PDAC. We aimed to assess whether cancer cells that stain positive for amylase and CK19 (ADM-like cancer cells) are present in human resected PDAC and to investigate their role in tumor progression.
Methods: We immunohistochemically investigated the presence of ADM-like cancer cells, and compared the clinical and histological parameters of PDAC patients with and without ADM-like cancer cells.
Results: ADM-like cancer cells were detected in 16 of 60 (26.7%) PDAC specimens. Positive staining for anterior gradient protein 2 (AGR2) was observed in 14 of 16 (87.5%) PDAC specimens with ADM-like cancer cells. On the other hand, the intensity of AGR2 expression (negative, low/moderate or high) was lower in PDAC with ADM-like cancer cells (9/7) than in PDAC without these cells (11/33) ($P = 0.032$). The presence of ADM-like cancer cells was significantly correlated with increased cell proliferation ($P = 0.012$) and tended to be associated with MUC1 expression ($P = 0.067$).
Conclusions: These results indicated that acinar cells may act as the origin of human PDAC, and that their presence may be useful for the stratification of human PDAC to predict prognosis.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most lethal among the 15 most common human cancers in the United States, with a 5-year survival rate of 11% [1]. PDAC is the fourth leading cause of cancer-related mortality in the United States and is projected to become the second leading cause of cancer-related death in the United States and Europe by 2030 [2]. This poor prognosis of human PDAC in part results from diagnosis at a late stage. Therefore, understanding the early transformative processes that lead to the development of PDAC is critical to discover biomarkers for the detection and intervention of PDAC at an early stage.

PDAC has been historically considered to originate from duct cells based on its morphology, i.e., the occurrence of dysplasia in putative preneoplastic ductal lesions and the absence of acinar dysplasia in patients with PDAC. Human pancreatic tumor histology has previously implicated the ductal compartment in PDAC [3]. However, whether pancreatic duct cells can give rise to PDAC remains controversial. Adult mouse duct cells do not develop PDAC with activation of mutant K-Ras only [4–6]. In addition to oncogenic K-Ras expression, a cellular transformation-permissive inflammatory environment appears to be imperative to initiate pancreatic carcinogenesis [7]. Although ductal and stem-like centroacinar cells are refractory to oncogenic transformation [8], an intermediate phenotype referred to as acinar-to-ductal metaplasia (ADM) is induced in the presence of acute or chronic inflammation [9,10], and some of the metaplastic lesions can progress into pancreatic intraepithelial neoplasia (PanIN) lesions or PDAC

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[11–18]. The process of pancreatic carcinogenesis has been confirmed exclusively in genetically engineered mouse models. However, little is known about whether acinar cell plasticity contributes to carcinogenesis in human PDAC, even though adult human pancreatic acinar cells are highly transformation-permissive [19,20]. Recently, cancer-associated ADM-like lesions that stained positive for amylase (acinar cell phenotype) and cytokeratin 19 (CK19) (duct cell phenotype) were demonstrated to be present in the invasive front of resected pancreatic cancer tissues [21]. To examine the possible role of acinar cell plasticity in the development of human PDAC, we aimed to assess whether cancer cells that stain positive for amylase and CK19 (ADM-like cancer cells) are present in human resected PDAC tissues and to investigate their roles in tumor progression.

2. Materials and methods

2.1. Human PDAC tissue

Formalin-fixed, paraffin-embedded surgical samples were obtained from 73 patients who underwent pancreatic resection for PDAC (n = 60), malignant tumors other than PDAC (ampullary cancer in 6, biliary tract cancer in 5, and duodenal cancer in 1) or chronic pancreatitis (n = 1) at Kawasaki Medical School Hospital from April 2010 to March 2021. Thus, 12 normal pancreatic tissues and one benign pancreatic lesion were used as control. The study protocol conformed to the 1975 Helsinki Declaration and was approved by the Ethics Committee of Kawasaki Medical School (admission No: 3613). The need for informed consent was waived by the Research Ethics Committee, because the study was retrospective and some patients had already been dead. PDAC was independently diagnosed by two experienced pathologists based on the histology of resected pancreatic tumors.

2.2. Clinical, biochemical, and histological parameters

All patients with PDAC were followed up after operation and survival time was defined as the interval between the diagnosis of PDAC and death or the last visit to the outpatient clinic up to December 31, 2021. Recurrence of PDAC after operation was diagnosed by contrasted computed tomography. The clinical stage was determined based on the TNM classification of the Union for International Cancer Control. When cancer cells with different histological differentiation were observed in the same PDAC specimen, the more poorly differentiated cells were adopted for the diagnosis of cancer histological differentiation.

2.3. Immunohistochemistry

Tissues were cut into 4-μm-thick sections and then subjected to hematoxylin and eosin (H&E) staining. Immunohistochemistry was performed using a rabbit anti-human alpha amylase polyclonal antibody (A8273, Sigma-Aldrich, St. Louis, MO; 1:500 dilution), a rabbit anti-human trypsin monoclonal antibody (EPR19498, Abcam, Cambridge, UK; 1:100 dilution), a rabbit anti-human pancreatic lipase monoclonal antibody (EPR6275, Abcam; 1:250 dilution), a mouse anti-human CK19 monoclonal antibody (NCL-CK19, Leica Biosystems, Wetzlar, Germany; 1:100 dilution), a rabbit anti-human anterior gradients protein 2 (AGR2) polyclonal antibody (HPA007912, Sigma-Aldrich; 1:250 dilution), or a mouse anti-human BCL10 monoclonal antibody (sc-5273, Santa Cruz Biotechnology, Dallas, TX; 1:100 dilution), or a mouse anti-human MUC-1 glycoprotein monoclonal antibody (NCL-MUC-1-CORE, Leica Biosystems, Wetzlar; 1:100 dilution), followed by incubation with secondary antibody (VENTANA OptiView DAB universal kit; Roche Diagnostics, Tokyo, Japan).

Fiji, an open-source platform for biological-image analysis [22], was used to quantify the mean percent area of positive staining for AGR2 in 3 randomly selected fields of view from digital images of each PDAC specimen. The proportion of AGR2-positive cells was classified as follows: low (<50%), moderate (50%≤ ~ <70%), and high (≥70%). A Ki67-positive nuclei labeling index was determined by a random evaluation of 1000 cells selected from several areas.

2.4. Statistical analysis

Baseline continuous variables are expressed as the mean ± standard deviation. Comparisons between two groups were carried out using the *t-test* for continuous variables, and the χ^2-test for categorical variables. Multivariate analysis of factors associated with amylase-positive PDAC was assessed using the logistic regression test. The overall survival rate and progression free survival rate were calculated using the Kaplan-Meier method, and differences among the groups were analyzed with the log-rank test. A *P* value of less than 0.05 was considered to indicate statistical significance. All analyses described above were performed using SPSS software (version 25; SPSS, Chicago, IL).

3. Results

3.1. Patients

The clinical and histological characteristics of the 60 patients (38 male, 22 female) are summarized in Table 1. The mean age of patients was 70.4 years (range, 43–89 years). The clinical PDAC stage was stage 0 in 2, stage IA in 5, stage IB in 4, stage IIA in 13, stage IIB in 19, and stage III in 17 patients. Histological differentiation of PDAC was well differentiated in 14, moderately differentiated in 36, and poorly differentiated in 10 patients. Pancreatitis in the tissue surrounding of PDAC was observed in 48 specimens (80%). Nine patients (15%) underwent neoadjuvant chemotherapy, and 53 patients (88%) underwent adjuvant chemotherapy. Response to neoadjuvant chemotherapy based on Response Evaluation Criteria in Solid Tumors (RECIST) (version1.1) was partial response (PR) in 8 and stable disease (SD) in one patient. Response to adjuvant chemotherapy based on RECIST version 1.1 was complete response (CR) in 19 and progressive disease (PD) in 34 patients. The outcome of the patients on December 31, 2021 was no recurrence in 21, recurrence in 13, death in 24, and unknown in 2 patients. The mean overall survival was 1153 ± 868 days.

Table 1
Clinical characteristics of the analyzed patients with pancreatic ductal adenocarcinoma (PDAC).

Clinical characteristic	Measurement(s)
Age at operation (years) (mean ± SD)	70 ± 10
Sex (male/female)	38/22
PDAC stage (0/IA/IB/IIA/IIB/III)	2/5/4/13/19/17
Histological differentiation (well/moderately/poorly)	14/36/10
Pancreatitis in the tissue surrounding PDAC (+/−)	48/12
Neoadjuvant chemotherapy (NAC) (+/−)	9/51
Response to NAC (CR/PR/SD/PD)	0/8/1/0
CA19-9 prior to NAC or resection (U/mL) (mean ± SD)	444.6 ± 1358.9
Adjuvant chemotherapy (+/−)	53/7
Response to adjuvant chemotherapy (CR/PR/SD/PD)	19/0/0/34
Outcome (no recurrence/recurrence/death/unknown)	21/13/24/2
Overall survival (days) (mean ± SD)	1153 ± 868

SD, standard deviation; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

3.2. Immunohistochemical assessment of resected PDAC specimens

To rule out pancreatic acinar cell carcinoma which produces pancreatic digestive enzymes, we immunohistochemically confirmed that BCL10 was not expressed in any of the resected PDAC samples, except for acinar cells in the tissue surrounding PDAC (Fig. 1). BCL10 was originally identified as a recurrent t (1; 14) (p22; q32) translocation in mucosa-associated lymphoid tissue (MALT) B-cell lymphoma [23], and has been reported to be a useful marker for pancreatic acinar cell carcinoma [24]. Then, we immunohistochemically assessed the expression of digestive enzymes such as amylase, trypsin, and lipase in the resected PDAC specimens to determine the pancreatic acinar cell phenotype. The cancer cells were positive for amylase in 16 PDAC specimens (26.7%), positive for trypsin in 6 PDAC specimens (10.0%), and negative for lipase in all specimens (Table 2). We also assessed the expression of CK19 to determine the pancreatic ductal phenotype. The cancer cells were positive for CK19 in all PDAC specimens. In addition, we examined the expression level of AGR2, which was reported to be significantly higher in duct-derived pancreatic cancer than in acinar-derived pancreatic cancer in genetically engineered mouse models [11]. AGR2 expression was detected in 95% (57/60) of the PDAC specimens, with the intensity being low in 17, moderate in 34, and high in 6 specimens (Table 2). In contrast, pancreatic ductal cells were negative for amylase and AGR2 in normal pancreatic tissues and benign pancreatic lesion. Consequently, on the basis of a combination of CK19, amylase, or AGR2 expression, the specimens were classified into the following four groups: group A: CK19 positive/amylase negative/AGR2 positive (43/60 72%), group B: CK19 positive/amylase negative/AGR2 negative (1/60, 2%), group C: CK19 positive/amylase positive/AGR2 positive 14/60, 23%), and group D: CK19 positive/amylase positive/AGR2 negative (2/60, 3%). We further immunohistochemically examined the MUC1 expression to assess the association of ADM-like cancer cells (cancer cells stained positive for amylase and CK19) with cancer promoting molecules in PDAC, since aberrant MUC1 expression has been reported to increase gradually with the formation of invasive carcinoma [25,26]. MUC1 was positively stained in 26.7% (16 of 60) of the PDAC specimens. (Fig. 1, Table 2). The mean percentage of specimens for which the cancer cell nuclei stained positively for Ki67 was 24 ± 2% (Table 2).

3.3. Comparative analysis of PDAC patients with and without ADM-like cancer cells

To clarify the characteristics of PDAC with ADM-like cancer cells, we compared the clinical and histological parameters of PDAC patients with and without ADM-like cancer cells. PDAC with ADM-like cancer cells showed lower histological differentiation ($P = 0.015$), a higher Ki67 labeling index (<20%/≥ 20%) ($P < 0.001$), higher trypsin expression ($P < 0.001$), lower AGR2 expression (negative to low/moderate to high, $P = 0.032$), and higher MUC1 expression ($P = 0.005$) (Table 3). Multivariate analysis identified a higher Ki67 labeling index (≥20%) ($P = 0.012$) as a factor that was significantly associated with the presence of ADM-like cancer cells. The expression of MUC1 also tended to be associated with the presence of ADM-like cancer cells, although this association did not reach statistical significance ($P = 0.067$) (Table 4). However, the overall survival rate and progression-free survival rate were similar between PDAC patients with and without ADM-like cancer cells

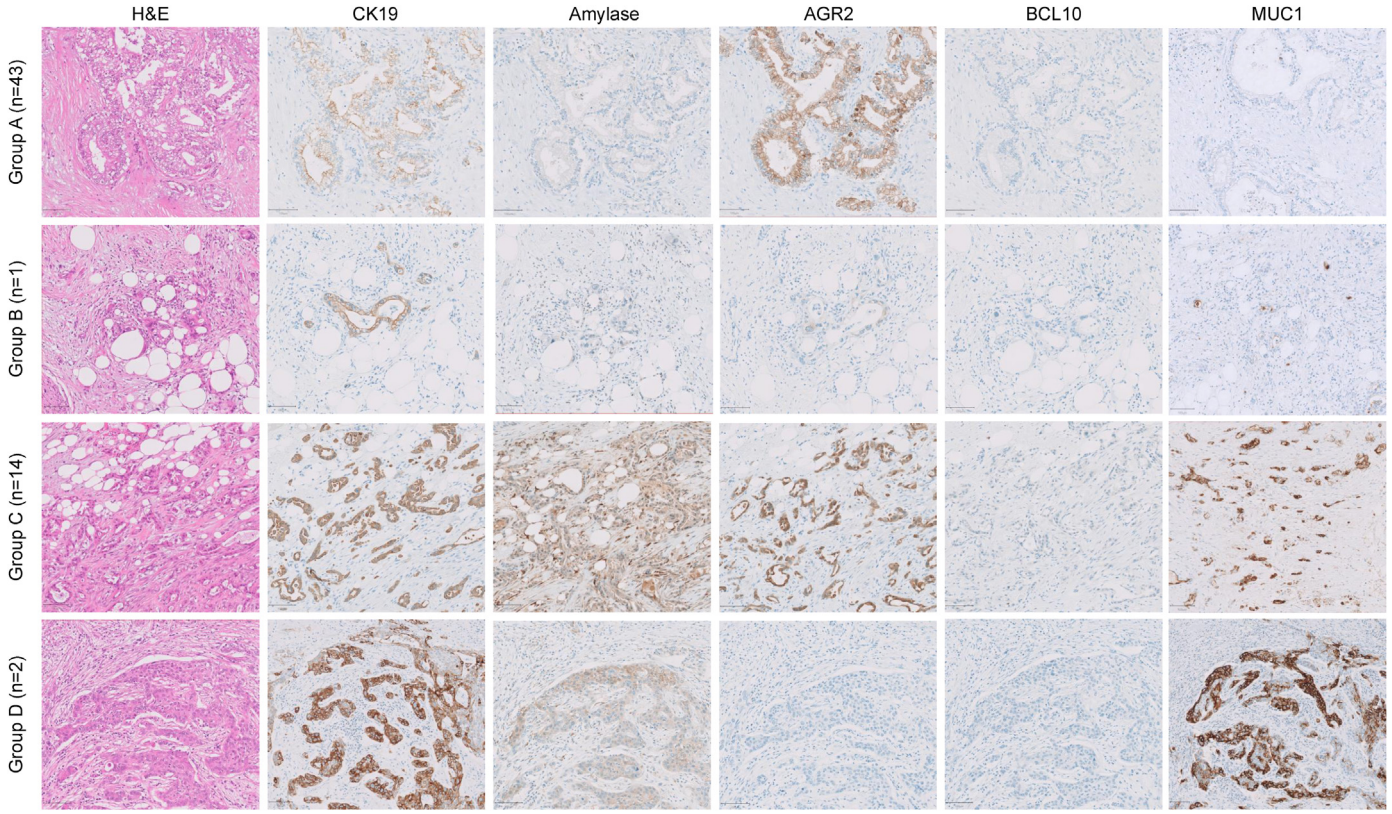

Fig. 1. Representative micrographs showing H&E, CK19, amylase, anterior gradient protein 2 (AGR2), BCL10, and MUC1 staining of operated human pancreatic ductal adenocarcinoma (PDAC) specimens. Scale bars represent 100 μm. Group A, CK19 positive/amylase negative/AGR2 positive; Group B, CK19 positive/amylase negative/AGR2 negative; Group C, CK19 positive/amylase positive/AGR2 positive; Group D, CK19 positive/amylase positive/AGR2 negative.

Table 2
Immunohistochemical analysis of operated pancreatic ductal adenocarcinoma (PDAC).

Pt	Age	Sex	Differentiation	CK19	Amylase	Trypsin	Lipase	AGR2	MUC1	Ki67 (%)
1	76	F	poorly	pos	pos	pos	neg	neg	pos	26.9
2	85	F	poorly	pos	pos	pos	neg	neg	neg	51.2
3	47	M	poorly	pos	pos	pos	neg	low	neg	46.9
4	61	M	poorly	pos	pos	neg	neg	low	pos	26.4
5	49	M	poorly	pos	pos	neg	neg	moderate	neg	14.3
6	89	M	poorly	pos	pos	neg	neg	moderate	pos	34.1
7	60	M	moderately	pos	pos	pos	neg	low	pos	21.2
8	78	M	moderately	pos	pos	pos	neg	low	neg	62.9
9	63	F	moderately	pos	pos	neg	neg	moderate	pos	15.8
10	76	F	moderately	pos	pos	neg	neg	low	pos	49.2
11	69	M	moderately	pos	pos	neg	neg	low	pos	27.7
12	72	M	moderately	pos	pos	neg	neg	low	neg	27.4
13	76	M	moderately	pos	pos	neg	neg	moderate	pos	34.1
14	80	F	moderately	pos	pos	neg	neg	moderate	pos	46.4
15	77	F	moderately	pos	pos	neg	neg	moderate	neg	35.7
16	73	M	well	pos	pos	pos	neg	moderate	neg	58.8
17	72	M	poorly	pos	neg	neg	neg	neg	neg	7.4
18	68	M	poorly	pos	neg	neg	neg	low	neg	18.7
19	68	F	poorly	pos	neg	neg	neg	moderate	pos	16.4
20	69	M	poorly	pos	neg	neg	neg	high	pos	55.2
21	67	M	moderately	pos	neg	neg	neg	moderate	neg	13.0
22	78	M	moderately	pos	neg	neg	neg	high	pos	41.2
23	81	F	moderately	pos	neg	neg	neg	high	neg	3.8
24	80	M	moderately	pos	neg	neg	neg	moderate	neg	10.6
25	54	M	moderately	pos	neg	neg	neg	moderate	neg	61.6
26	78	M	moderately	pos	neg	neg	neg	moderate	neg	4.5
27	73	F	moderately	pos	neg	neg	neg	low	neg	9.6
28	55	M	moderately	pos	neg	neg	neg	moderate	pos	50.6
29	87	M	moderately	pos	neg	neg	neg	low	neg	64.5
30	69	F	moderately	pos	neg	neg	neg	moderate	neg	6.9
31	69	F	moderately	pos	neg	neg	neg	moderate	neg	7.1
32	68	M	moderately	pos	neg	neg	neg	high	neg	30.5
33	63	M	moderately	pos	neg	neg	neg	moderate	neg	3.8
34	64	M	moderately	pos	neg	neg	neg	moderate	neg	1.4
35	66	F	moderately	pos	neg	neg	neg	moderate	neg	3.2
36	71	M	moderately	pos	neg	neg	neg	moderate	neg	16.3
37	63	F	moderately	pos	neg	neg	neg	moderate	neg	23.4
38	79	M	moderately	pos	neg	neg	neg	moderate	pos	30.6
39	65	M	moderately	pos	neg	neg	neg	high	neg	43.1
40	79	M	moderately	pos	neg	neg	neg	high	pos	15.9
41	82	F	moderately	pos	neg	neg	neg	moderate	pos	15.2
42	75	M	moderately	pos	neg	neg	neg	moderate	neg	38.2
43	80	F	moderately	pos	neg	neg	neg	moderate	neg	21.7
44	72	F	moderately	pos	neg	neg	neg	low	neg	14.5
45	70	M	moderately	pos	neg	neg	neg	low	neg	8.3
46	70	F	moderately	pos	neg	neg	neg	low	neg	10.5
47	78	M	moderately	pos	neg	neg	neg	moderate	neg	14.9
48	51	F	well	pos	neg	neg	neg	moderate	neg	4.9
49	61	M	well	pos	neg	neg	neg	moderate	neg	2.1
50	63	F	well	pos	neg	neg	neg	low	neg	32.0
51	83	F	well	pos	neg	neg	neg	moderate	neg	4.6
52	68	M	well	pos	neg	neg	neg	low	neg	4.3
53	72	F	well	pos	neg	neg	neg	low	neg	6.3
54	65	M	well	pos	neg	neg	neg	low	neg	20.9
55	72	F	well	pos	neg	neg	neg	moderate	neg	14.7
56	82	M	well	pos	neg	neg	neg	moderate	neg	6.3
57	73	M	well	pos	neg	neg	neg	moderate	neg	4.7
58	88	M	well	pos	neg	neg	neg	moderate	neg	43.7
59	43	M	well	pos	neg	neg	neg	moderate	neg	9.2
60	59	M	well	pos	neg	neg	neg	moderate	neg	44.8

AGR2, anterior gradients protein 2; pos, positive; neg, negative; low, positive cells <50%; moderate, 50%≤ positive cells <70%; high, positive cells ≥70%.

(Fig. 2). Unfortunately, we could not compare the clinical and histological parameters of patients with AGR2 expression and those without among PDAC patients with ADM-like cancer cells, since only 2 PDAC patients with ADM-like cancer cells did not show AGR2 expression.

4. Discussion

Differentiated acinar cells are characterized by expression of the transcription factors, PTF1A, MIST1, GATA4, and NR5A2, which regulate the expression of digestive enzymes such as amylase and elastase [27–29]. Thus, the expression of amylase is recognized as a pancreatic acinar cell phenotype. Acinar cells are sensitive to experimental injury or stress, losing their normal phenotype [30]. When purified acinar cells are cultured, they become negative for amylase and gain ductal features such as CK19 expression over a few days [31]. Pancreatic acinar cells can dedifferentiate or transdifferentiate to an embryonic progenitor phenotype in which they

Table 3
Comparison of clinical and histological characteristics between pancreatic ductal adenocarcinoma (PDAC) patients without ADM-like lesions and those with ADM-like lesions.

Clinical or histological characteristic	PDAC patients without ADM-like cancer cells	PDAC patients with ADM-like cancer cells	*P* value
Number	44	16	
Age at operation (years) (mean ± SD)	70 ± 9	71 ± 12	0.895
Sex (male/female)	28/16	10/6	0.583
PDAC stage (0/IA/IB/IIA/IIB/III)	2/4/3/8/15/12	0/1/1/5/4/5	0.829
CA19-9 prior to NAC or resection (U/mL) (mean ± SD)	445.8 ± 1420.5	441.1 ± 1216.1	0.991
Neoadjuvant chemotherapy (NAC) (+/−)	6/38	3/13	0.449
Response (CR + PR) rate of neoadjuvant chemotherapy (%)	83% (5/6)	100% (3/3)	1.00
Adjuvant chemotherapy (+/−)	37/7	16/0	0.099
Disease control (CR + PR + SD) rate of adjuvant chemotherapy (%)	41% (15/37)	25% (4/16)	0.441
Pancreatitis in tissue surrounding PDAC (absent/present)	11/33	1/15	0.103
Histological differentiation (well/moderately/poorly)	13/27/4	1/9/6	0.015
Ki67 labeling index (<20%/≥ 20%)	29/15	2/14	<0.001
Trypsin (negative/positive)	44/0	10/6	<0.001
AGR2 (negative or low/moderate or high)	11/33	9/7	0.032
MUC1 expression (positive/negative)	7/37	9/7	0.005

PDAC, pancreatic ductal adenocarcinoma; AGR2, anterior gradients protein 2; CR, complete response; PR, partial response; SD, stable disease.

Table 4
Factors associated with amylase-positive pancreatic ductal adenocarcinoma (PDAC) in patients with PDAC who underwent pancreatectomy.

Variables	Univariate	Multivariate		
	P value	Odds ratio	95% CI	*P* value
Ki67 labeling index (≥20%)	0.001	10.203	1.65-63.13	0.012
Poorly differentiated carcinoma	0.015	4.443	0.62-31.66	0.137
Low AGR2 expression	0.028	3.678	0.73-18.56	0.115
MUC1 expression	0.003	4.675	0.90-24.39	0.067
Trypsin expression	0.999	–	–	–

AGR2, anterior gradients protein 2.

express ductal markers in the process of ADM [20,32,33], which is believed to contribute to the regeneration of acinar structures and repopulation of the pancreas. Many recent studies in rodents have shown evidence that acinar cells are the cells of origin for PDAC [11–16]. In fact, chronic or repetitive acute pancreatitis induces dedifferentiation in acinar cells, which become sensitive to neoplastic transformation [17,18]. However, the cell origin of human PDAC is less evident.

In the present study, we have shown that a subpopulation of human PDAC expresses amylase and CK19. This finding can be interpreted in at least two ways. First, PDAC may have developed via ADM and retained the phenotype of acinar cells (amylase expression). Second, duct cells may have transdifferentiated and acquired an acinar cell phenotype in the process of PDAC development. Although a recent single-cell RNA sequencing study revealed high heterogeneity within the duct cell population, which includes cells undergoing transition from a ductal to an acinar phenotype [34,35], much less is known about the possible role of duct cells in phenotypic plasticity in PDAC development. Additionally, duct cells are far outnumbered by acinar cells, suggesting that they are stochastically less prone to tumor development. AGR2 was reported to be upregulated in regeneration of the acinar cell compartment and ADM-to-neoplastic transformation in a genetically engineered pancreatitis-associated PDAC mouse model [36]. The high proportion (14/16, 87.5%) of AGR2 positivity among PDAC patients with ADM-like cancer cells appears to be consistent with upregulation of AGR2 in ADM-to-neoplastic transformation in the genetically engineered pancreatitis-associated PDAC mouse model. On the other hand, the finding that AGR2 expression intensity (negative or low/moderate or high) was lower in PDAC with ADM-like cancer cells (9/7) than in PDAC without (11/33) ($P = 0.032$) is compatible with the low AGR2 expression in acinar-derived tumors and high AGR2 expression in duct-derived tumors reported in genetically engineered PDAC mouse models [11]. Also, a possible role of neoadjuvant chemotherapy in the development of ADM-like cancer cells can be excluded, since both neoadjuvant chemotherapy rate (3/13 [23%] vs. 6/38 [16%]) and its effect (3/3 [100%] vs. 5/6 [83%]) were similar in PDAC patients with and without ADM-like cancer cell (Table 3). Taken together with these results, the presence of ADM-like cancer cells suggests a possible role of ADM-to-neoplastic transformation in human PDAC development. However, we cannot exclude the possibility that a subpopulation of PDAC without amylase expression developed via ADM, if the phenotypes of acinar cells, such as amylase expression, had been lost in the process of acinar cell transdifferentiation into duct-like cells. However, the mechanisms by which ADM progresses to PDAC remains unclear. In this respect, AGR2-dependent nuclear import of RNA polymerase II has been reported to prevent the ataxia telangiectasia mutated and Red 3-related (ATR)-mediated passive p53 activation on the incipient acquisition of neoplastic phenotype in ADM lesions (36). Upregulation of AGR2 was confirmed in ADM-like cancer cells in this study as well as ADM lesions in the genetically engineered PDAC mouse model (36). These results provide one of molecular mechanisms underlying transition from ADM to neoplastic formation in human PDAC development.

Interestingly, the presence of ADM-like cancer cells was significantly correlated with a high level of cell proliferation. To the best of our knowledge, we have demonstrated for the first time that PDAC with ADM-like cancer cells is associated with high cell proliferation. Although a lineage tracing study of human acinar cells indicated that human acinar cells undergo a transdifferentiation, namely conversion from a differentiated cell type into another differentiated cell type when cultured as monolayers [20,37], acinar cells were also reported to dedifferentiate prior to PDAC development in *Kras* mutation-driven PDAC mouse models [17,18,38]. Additionally, cancer-associated ADM-like lesions have been reported to be present in the invasive front of PDAC and to exhibit upregulation of pancreatic cancer-related genes [21]. Consistent with these results, it makes sense that PDAC with ADM-like cancer cells displayed higher cell proliferation and MUC1 expression, although the difference in MUC1 expression did not reach statistical significance ($P = 0.067$). However, further studies are required to clarify why the presence of ADM-like cancer cells was associated with higher proliferation and/or MUC1 expression. Although these results are seemingly contradictory to the similar overall survival rate and progression-free survival rate in PDAC patients with and without ADM-like cancer cells, the response to adjuvant chemotherapy may have affected patient outcome since most patients (88%) underwent adjuvant chemotherapy, with 84% of PDAC

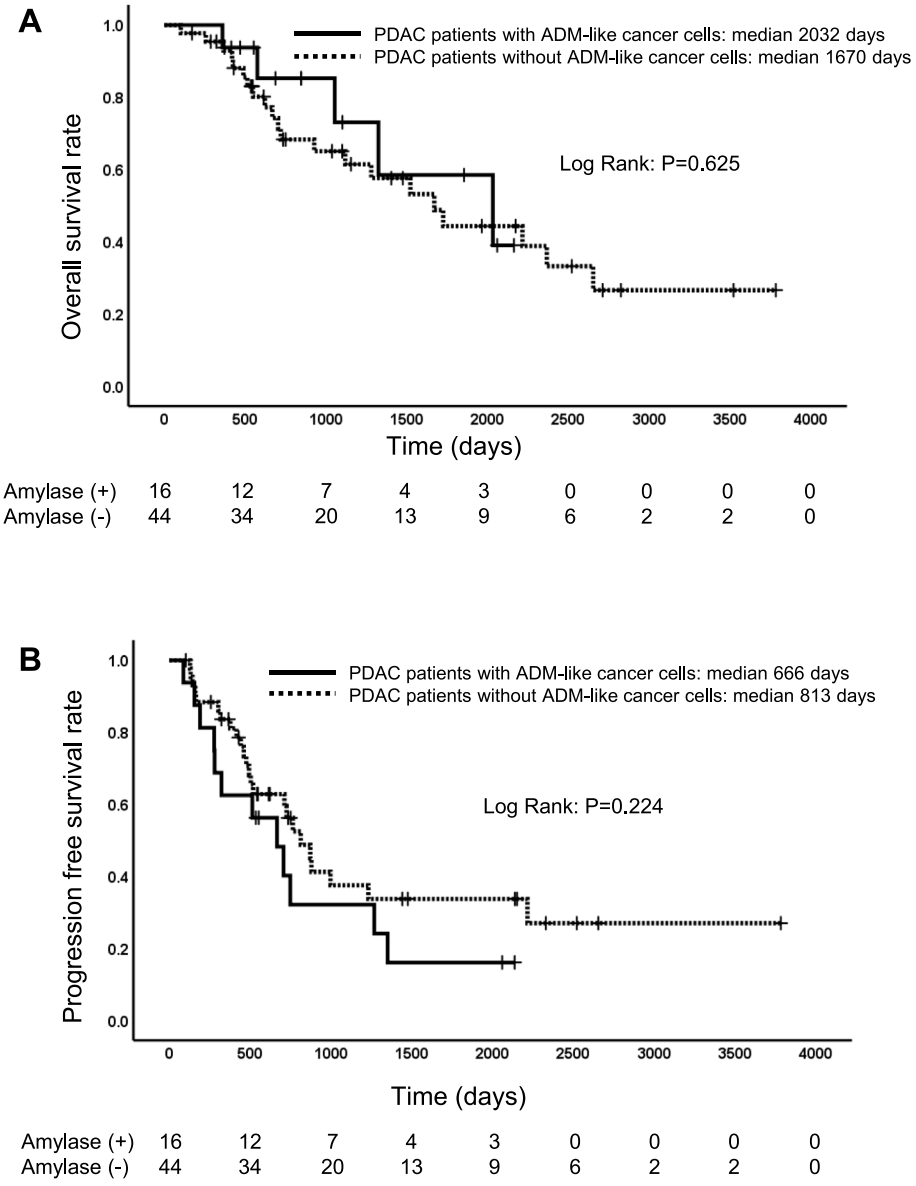

Fig. 2. Overall survival rate and progression-free survival rate of pancreatic ductal adenocarcinoma (PDAC) patients with acinar-to-ductal metaplasia (ADM)-like cancer cells and those without. (A) Overall survival rate of PDAC patients with ADM-like cancer cells (solid line) and those without (dotted line). (B) Progression-free survival rate of PDAC patients with ADM-like cancer cells (solid line) and those without (dotted line).

patients without ADM-like cancer cells and 100% of PDAC patients with ADM-like cancer cells undergoing adjuvant chemotherapy.

In conclusion, we have shown that ADM-like cancer cells are present in a subset of patients with PDAC, which indicates the possibility of acinar cells as the origin of human PDAC. Additionally, the presence of ADM-like cancer cells in human PDAC was associated with higher cell proliferation. These results may be useful for the stratification of human PDAC to predict patient prognosis or develop therapeutic strategies.

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