

The Prognostic Role of CD73/A2AR Expression and Tumor Immune Response in Periapillary Carcinoma Subtypes

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Abstract

Introduction: Periapillary adenocarcinoma (PAAC) is a rare, lethal heterogeneous group of malignancy that differs in their molecular phenotypes. Ecto-5'-nucleotidase (CD73)/adenosine A2A Receptor (A2AR) pathway has shown an emerging role in cancer therapy through modulating the immune response. Therefore, this study aimed to explore the functional role of CD73 and A2AR in pancreatic ductal adenocarcinoma (PDAC) and ampullary carcinoma (AC). **Material and methods:** An immunohistochemical study for CD73 and A2AR carried on 48 PDAC cases, 21 AC cases and 34 adjacent non-tumor tissues that were taken from the farthest point of normal pancreatic tissue away from the tumor. **Results:** CD73 was overexpressed in the PDAC ($p < 0.001$), and AC ($p = 0.004$) groups compared to their non-tumor tissues. However, A2AR was overexpressed in the PDAC group ($p = 0.003$) but not in the AC group ($p = 0.359$) compared to non-tumor tissue. In the PDAC group, CD73 overexpression was significantly associated with longer overall survival ($p = 0.018$). In contrary, A2AR overexpression was significantly associated with high grade ($p = 0.001$) and late-stage ($p = 0.01$). Both markers had no prognostic impact on AC. In the meantime, tumor immune response showed a negative prognostic role in PDAC and AC. The prognostic role of tumor immune response in the PDAC group was strongly modulated by CD73 and A2AR expression. **Conclusions:** PDAC and AC shared CD73 Overexpression while A2AR was overexpressed in PDAC only. In PDAC, CD73 and A2AR showed an opposed prognostic effect but both had no prognostic impact on AC. In addition, tumor immune response showed a controversial impact on the prognosis of PDAC and AC.

Keywords: A2AR- ampullary carcinoma- CD73- pancreatic ductal carcinoma- prognosis

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Introduction

The incidence of periampullary adenocarcinomas (PAACs) is increasing worldwide and accounts for 0.2% of all gastrointestinal tract tumors (Siegel et al., 2020). PAACs arise from four anatomical sites: the head of the pancreas (50%–75%), the ampulla of Vater (10%–20%), the distal common bile duct (10%–20%), and the second part of the duodenum (3%–7%) (Hester et al., 2019). In Egypt, pancreatic ductal adenocarcinoma (PDAC) and ampullary carcinoma (AC) constitute 15.9% and 1.8%, respectively, of all hepatobiliary and gastrointestinal malignancies (Mokhtar et al., 2016). Risk factors for PDAC include smoking, diabetes mellitus, alcohol intake, chronic and hereditary pancreatitis, and familial cancer syndromes. Duodenal carcinoma and AC may arise from preexisting adenomas, and bile duct cancers are associated with primary sclerosing cholangitis and congenital anomalies (Hutchins and Williamson, 2003).

Radical pancreaticoduodenectomy (PD) is the treatment of choice for all resectable PAAC subtypes.

However, even after radical resection, the survival rate among patients with PAAC cancers varies greatly, with PDAC having the lowest overall survival (OS) rate (Ferchichi et al., 2018). Several explanations for these variations exist, including the onset of clinical presentation, tumor growth pattern, etiopathological factors, and resistance to several chemotherapeutic agents (Hester et al., 2019; Rawla et al., 2019). In addition, several studies have reported a variation in the molecular and genetic alterations. This could influence tumor prognosis and offer a potential individualized therapeutic target for each subtype (Sikdar et al., 2018).

CD73 (ecto-5'-nucleotidase) is an ectoenzyme that catalyzes the rate-limiting step in extracellular adenosine and participates in cell adhesion (Antonioli et al., 2016). Under physiological conditions, CD73 is expressed by stromal cells, follicular dendritic cells, endothelial cells, and some immune cells (Bono et al., 2015). Once activated by inflammation and hypoxia, CD73 releases extracellular adenosine that binds to specific G-protein-coupled receptors (A1, A2A, A2B, and A3) (Antonioli et al., 2016).

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Adenosine A2A Receptor (A2AR), the most predominant form, is expressed in immune cells and serves as an anti-inflammatory barrier (Haskó et al., 2008). The main role of CD73 and A2AR in carcinogenesis is through modulation of tumor immune response (Vigano et al., 2019). Chronic inflammation-induced cancer is one of the cancer hallmarks as chronic inflammation participates in tumor progression, genomic instability, enhancing angiogenesis, and inhibition of tumor apoptosis. However, previous studies reported a dual role of tumor immune response, mainly T-lymphocytes, in cancer progression and metastasis. Their role could be modulated by the tumor stage, initiation versus advanced, and the type of tumor immune cells and subtype of T-cells activated, CD4, CD8 or regulatory T cells (Tregs) (Gonzalez et al., 2018). However, the prognostic role of CD73/A2AR in different human malignancies is a matter of controversy (Supernat et al., 2012; Wettstein et al., 2015; Monteiro et al., 2018; Yan et al., 2019).

Therefore, we aimed to study the difference between PDAC, and AC in the context of clinicopathological parameters, the pathogenic role of CD73 and A2AR, and the impact of tumor immune response in modulating the prognosis for each group.

This could identify a possible signature in classifying PAAC and individualize selective target therapies.

Materials and Methods

This is a retrospective study involving 69 patients with PAAC divided into two subgroups: the PDAC (n = 48) and AC (n = 21) groups. Data were collected from 2016 to 2019. Moreover, this study included 34 cases of adjacent non-tumor tissues (23 pancreatic and 11 intestinal tissues) as the control group. The patients presented with obstructive jaundice and were assessed thoroughly for resectability. All cases underwent PD surgery at the Hepatopancreatobiliary Department. Patients underwent exploration through bilateral subcostal or midline incision followed by Whipple's or pylorus -preserving pancreaticoduodenectomy (PPPD). Pancreatic reconstruction was done by either pancreaticojejunostomy or pancreaticogastrostomy. The patients' clinical, laboratory, and OS data were collected from the patients' medical records. OS data were calculated in months from the date of diagnosis to the time of death or the date of the last follow-up visit (for at least 12 months after surgery).

Formalin-fixed, paraffin-embedded blocks for both tumor tissues and adjacent non-tumor tissues were obtained from PD specimens. Histopathological assessment of all malignant cases was performed according to the fifth edition of the World Health Organization's classifications of digestive tract tumors and the eighth edition of the American Joint Committee on Cancer's staging system (Chun et al., 2017; Nagtegaal et al., 2020). For statistical purposes, tumor cases were categorized into two-tiered pathological grades, low (GI and GII) and high (GIII), and two pathological stages, early (I and II) and late (III and IV). For tumor immune response, only the percentage of tumor-infiltrating mononuclear cells (TIMC), after

exclusion of neutrophils or macrophages, was assessed then cases were divided into low and high using the median (Hwang et al., 2016).

Tissue Microarray construction (TMA)

TMA blocks were manually prepared from tumor cases using a 2-mm tissue arrayer needle set (Breecher Instrument, USA) by SA. At least two representative viable tissue cores from the tumor tissues and one core from the non-tumor tissues.

Immunohistochemical staining

Two sections were cut from each TMA block and immunostained using the streptavidin–biotin-amplified system. The primary antibodies used were rabbit polyclonal anti-human CD73 diluted as 1:75 (Cat.#YPA1566) and anti-human A2AR diluted as 1:350 (Cat.#YPA2101) obtained from Chongqing Biopsies Co., Ltd, China. After deparaffinization and rehydration of the tissues, the antigens were retrieved using a high-PH ethylenediaminetetraacetic acid solution and cooled at room temperature. The slides were incubated overnight at 4°C with primary antibodies. The secondary antibody was applied using Ultravision's detection system anti-polyvalent horseradish peroxidase/3,3'-diaminobenzidine (DAB), ready-to-use, Neomarker, and staining was visualized using DAB and Mayer's hematoxylin as a counterstain. Positive control, stromal lymphocytes, and negative controls were included.

Antibody assessment methods

Positive expression of CD73 was considered if any tumor cells showed cytoplasmic and/or membranous brownish staining (Sciarra et al., 2019). The extent of CD73 staining was graded as follows: 0 when all tumor cells were negative, score 1 if less than a third of the tumor cells were positive, score 2 if more than a third of the tumor cells were positive, and score 3 if more than two-thirds of the tumor cells were positive (Ono et al., 2018). Histoscore (H score) was used to compare the tumor tissues and adjacent non-tumor tissues in a paired group (Inoue et al., 2017a).

Regarding the A2AR antibody, positive expression is considered if any tumor cells showed cytoplasmic and/or membranous brownish staining. The intensity of A2AR was scored as 0 (no staining), 1 (mild), 2 (moderate), or 3 (strong) (Mediavilla-Varela et al., 2013). H score was assessed, and the median was used as the cut-off point to divide the cases into low and high A2AR (Inoue et al., 2017b).

Statistical analysis

Data were collected, tabulated, and statistically analyzed using Statistical Package for the Social Sciences (version 20; IBM Corp., Armonk, NY, USA). The chi-square test was used to measure the association between the qualitative variables, and when more than 25% of the cells had an expected count of less than five, Fisher's exact was used. The Wilcoxon test was used to compare not normally distributed quantitative data for two paired groups. Pearson's correlation coefficient was used

to study the correlation between two variables. Two-tailed P-values are considered statistically significant when they are less than or equal to 0.05. The Kaplan–Meier plots and log-rank test were used to evaluate the patients’ OS data. Variables significantly related to the OS were then included in the multivariate Cox proportional hazard regression model.

Results

Clinicopathological data of the PAAC cases under study

This study included 69 cases of PAAC divided into two main groups: 48 (69.6%) cases of PDAC and 21 (30.4%) cases of AC. Grossly, all cases were solitary, and all PDAC cases were located in the head. Significant differences in tumor size, perineural invasion, and complete resectability of the mass were observed between PDAC and AC cases. PDAC cases had a significantly larger tumor size than AC cases ($p = 0.004$). In addition, perineural invasion was significantly present in 95.4% of PDAC compared to 52.4% of AC cases ($p < 0.001$). Resection of the tumor mass with clean surgical margin was significantly achieved in AC cases; however, only half of PDAC cases showed free resection margin ($p = 0.002$). The detailed clinicopathological data are shown in (Supplementary Table 1).

The expression of CD73 and A2AR in both the PAAC groups and adjacent non-tumor tissues is demonstrated in (Table 1)

There was a positive CD73 expression in all PDAC and AC cases. In non-tumor tissue, only 26% adjacent pancreatic and 36.4% of the intestinal tissues showed CD73 positive expression. Regarding A2AR expression, 95.8% and 100% of the PDAC and AC cases, respectively, showed positive expression. However, A2AR was expressed in 26% and 63.6 of non- tumor pancreatic and intestinal tissues, respectively. No statistically significant difference was found between the PDAC and AC groups regarding CD73 /A2ar expression (Table 1).

Comparative expression of CD73 and A2AR in both the PAAC groups and adjacent non-tumor tissues (Table 2)

The expression of CD73 was significantly higher in the PDAC and AC groups than that in the corresponding non-tumor tissues ($p < 0.001$ and $p = 0.004$, respectively) (Figure 1). Meanwhile, A2AR expression was significantly higher in the PDAC group compared to adjacent non-tumor tissue ($p = 0.003$) (Figure 2 a-b). However, no significant difference in A2AR expression was found between AC and adjacent non-tumor tissue ($p = 0.359$)

(Figure 2 c-d).

The association of CD73 and A2AR expression with clinicopathological parameters of both PAAC groups is shown in (Table 3)

There was no significant association between CD73 expression and the clinicopathological data in the PDAC group. However, A2AR overexpression was significantly associated with high pathological grade and late tumor stage ($p = 0.001$ and $p = 0.01$, respectively).

In AC cases, no significant association was observed between the expressions of CD73 and A2AR and clinicopathological parameters.

Correlation between CD73 and A2AR expression in both PAAC groups

No significant correlation was found between CD73 and A2AR in the PDAC group. However, a positive correlation between CD73 and A2AR was observed in the AC group ($r = 0.116$, $p = 0.434$ and $r = 0.466$, $p = 0.03$, respectively).

The impact of TIMC on the prognosis of the PDAC and AC groups and their association with CD73 and A2AR expressions

A significant association was observed between low TIMC levels and small tumor size in the PDAC group ($p = 0.029$). However, in the AC group, low TIMC levels showed a significant association with the positive perineural and lymph node invasion ($p = 0.002$ and $p = 0.03$, respectively). Regarding the association of the expressions of CD73 and A2AR with the immune responses in PAAC

Table 1. Comparative CD73 and A2AR Expression in PDAC and AC

Marker’s expression	PDCA (48 cases) N (%)	AC (21 cases) N (%)	Chi-square test
CD73 scoring			
Negative	0 (0)	0 (0)	1.136 $p = 0.57$
Score 1	8 (16.7)	2 (9.5)	
Score 2	13 (27.8)	8 (38.1)	
Score 3	27 (56.3)	11(52.4)	
A2AR intensity			
Negative	2 (4.2)	0 (0)	2.79 $p = 0.43$
Mild	13 (27.8)	9 (42.9)	
Moderate	23 (47.9)	7 (33.3)	
Strong	10 (20.8)	5 (23.8)	

PDAC, Pancreatic ductal adenocarcinoma; AC, Ampullary carcinoma; CD73, Ecto-5'-nucleotidase; A2AR, Adenosine A2A Receptor; **, highly significant.

Table 2. Comparative CD73 and A2AR Expression between Tumor and Adjacent Non-Tumor

Markers	PDAC	Non-tumor	Wilcoxon test	AC	Non-tumor	Wilcoxon test
CD73 H. score	152.8±77.7	3.9±8.4	-4.1 $p < 0.001^{**}$	137.6±74.1	17.7±30.8	-2.847 $P=0.004^{*}$
A2AR H. score	125.4±68.2	40.9±79.6	-2.9 $p = 0.003^{**}$	133±79.2	77±112.3	-0.918 $P=0.359$

PDAC, Pancreatic ductal adenocarcinoma; AC, Ampullary carcinoma; CD73, Ecto-5'-nucleotidase; A2AR, Adenosine A2A Receptor; **, highly significant.

Table 3. The Association of CD73 and A2AR with the Clinicopathological Parameters of PDAC

Variables	CD73 intensity			Chi-square	Variables	A2AR Intensity				Chi-square
	Score 1	Score 2	Score 3			Negative	Mild	Moderate	Strong	
	N (%)	N (%)	N (%)			N (%)	N (%)	N (%)	N (%)	
Tumor size										
≤4	3 (37.5)	3 (23.1)	15 (55.6)	3.914	≤4	2 (100)	5 (38.5)	12 (52.2)	2 (20)	5.674
>4	5 (62.5)	10 (67.9)	12 (44.4)	P = 0.141	>4	0 (0)	8 (61.5)	11 (47.8)	8 (80)	P = 0.13
Tumor grade										
Low	8 (100)	12 (92.3)	23 (85.2)	1.593	Low	1 (50)	13 (100)	23 (100)	6 (60)	16.92
High	0 (0)	1 (7.7)	4 (14.8)	p=0.45	High	1 (50)	0 (0)	0 (0)	4 (4)	p = 0.001**
Lymph vascular invasion										
Positive	1 (12.5)	4 (30.8)	10 (37)	1.731	Positive	1 (50)	5 (38.5)	6 (26.1)	3 (30)	0.94
Negative	7 (87.5)	9 (69.2)	17 (63)	p = 0.421	Negative	1 (50)	8 (61.5)	17 (73.9)	7 (70)	p = 0.82
Perineural invasion										
Positive	7 (87.5)	12 (92.3)	27 (100)	2.97	Positive	2 (100)	13 (100)	21 (91.3)	10 (100)	2.27
Negative	1 (12.5)	1 (7.7)	0 (0)	P = 0.227	Negative	0 (0)	0 (0)	2 (8.7)	0 (0)	p = 0.52
Lymph node metastasis										
Positive	4 (50)	9 (69.2)	21 (77.8)	2.327	Positive	2 (100)	9 (69.2)	15 (65.2)	8 (80)	1.59
Negative	4 (50)	4 (30.8)	6 (22.2)	P = 0.312	Negative	0 (0)	4 (30.8)	8 (34.8)	2 (20)	p = 0.66
Tumor stage										
Early	5 (62.5)	8 (61.5)	17 (63)	0.008	Early	2 (100)	8 (61.5)	18 (78.3)	2 (20)	11.35
Late	3 (37.5)	5 (38.5)	10 (37)	P=0.996	Late	0 (0)	5 (38.5)	5 (21.7)	8(80)	P = 0.01*
TIMC										
≤20	3 (37.5)	10 (76.9)	23 (85.2)	7.519	≤20	2 (100)	11 (84.6)	18 (78.3)	5 (50)	4.77
>20	5 (62.5)	3	4	P=0.023*	>20	0	2	5	5	P =

*, Significant; **, Highly significant; PDAC, Pancreatic ductal adenocarcinoma; CD73, Ecto-5'-nucleotidase; A2AR, Adenosine A2A Receptor.

cases, in the PDAC group, CD73 overexpression was significantly associated with low TIMC levels ($p = 0.023$) (Table 3). However, A2AR high H. score was significantly associated with high TIMC levels ($p = 0.03$) (data not tabulated). No significant association between CD73 and A2AR expressions and TIMC in the AC group ($p = 0.43$ and $p = 0.67$, respectively).

Survival analysis

The mean OS time of the patients with PDAC was 16.84 months (range, 11.83–21.85), whereas patients with AC had a mean OS time of 21.55 months (range, 13.37–29.77) with no significant difference between both groups ($p = 0.36$) (Table 4).

Univariate analysis for the 27 PDAC cases showed that short OS time was associated with hepatitis C virus (HCV) etiology, high serum CA19-9, and low CD73 expression ($p = 0.002$, $p = 0.057$, and $p = 0.014$, respectively). Furthermore, multivariate COX-regression analysis showed that low CD73 expression was the most independent prognostic factor affecting the patients' OS ($p = 0.018$).

Univariate analysis for 10 AC cases showed that short OS time was associated with positive perineural and lymph node invasions ($p = 0.054$ and $p = 0.008$, respectively). Furthermore, multivariate COX-regression analysis showed that lymph node invasion was the most independent prognostic factor affecting the patients' OS ($p = 0.026$).

Table 4 . Univariate OS Analysis of PDAC and AC Groups Classified by Clinicopathological and Immunohistochemical Parameters

Variables	Mean survival time (95% CI) of PDAC	P-value
Etiology		
HCV	5.5 (2.77-8.23)	0.002**
Non-viral	15.167 (10.836-19.498)	
CA19.9		
Low	11.4 (6.979-15.821)	0.057*
High	4.25 (0-9.64)	
CD73 score		
Positive in 1/3 of tumor cells	14.875 (5.737-24.013)	0.014*
Positive 1/3 to 2/3 of tumor cells	5.5 (1.967-9.033)	
Positive > 2/3 of tumor cells	19.5 (13.4-25.6)	
Variables	Mean survival time (95% CI) in AC	P-value
Perineural invasion		
Positive	15.357 (7.764-22.950)	0.054*
Negative	36 (0-36)	
Lymph node		
Positive	11.9 (2.905-20.895)	0.008**
Negative	31.2 (24.889-37.511)	

OS, Overall survival; SE, Standard error; PDAC, Pancreatic ductal adenocarcinoma; AC, Ampullary carcinoma; HCV, Hepatitis C virus; CD73, CD73, Ecto-5'-nucleotidase.

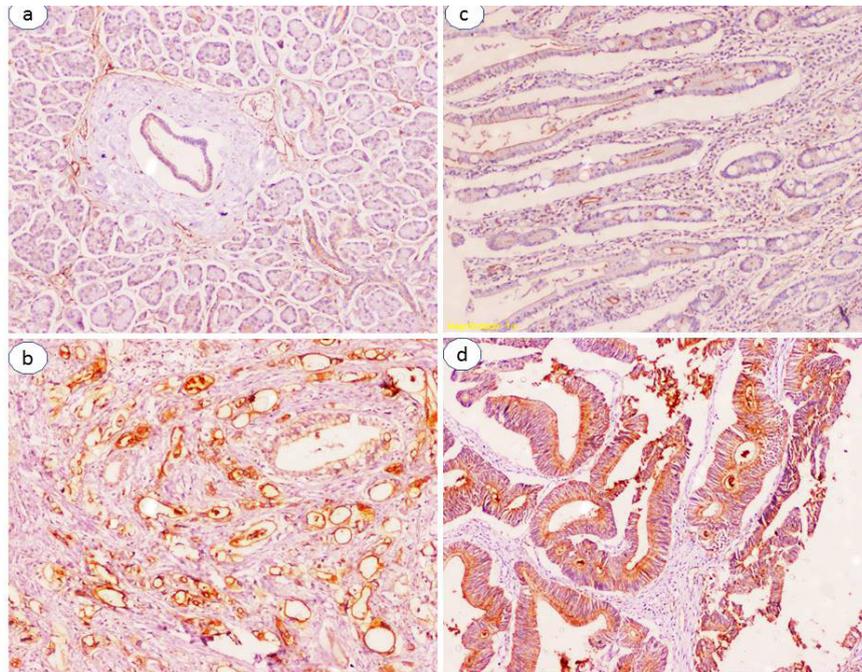


Figure 1. The Immunohistochemical Expression of CD73 in PDAC and AC. a) Low expression of CD73 in adjacent non-tumor pancreatic duct (x100), b) Strong expression of CD73 in PDAC (x200), c) Low expression of CD73 in adjacent non-tumor intestinal tissue (x100), d) Strong expression of CD73 in AC (x200),

Discussion

This study aimed to focus on clarifying the difference between PAAC subtypes, namely PDAC and AC with the clinicopathological and survival data and the functional role of CD73/A2AR activation. We further illustrated the impact of the tumor immune response on the prognosis of both tumors and its association with CD73/A2AR expression. The findings of this study showed significant

difference between PDAC and AC regarding the clinicopathological prognostic parameters. In addition, CD73 and A2AR functioned invariably in both groups. Despite CD73 and A2AR were overexpressed in PDAC, they showed an opposing prognostic effect. Furthermore, only CD73 had a role in the development of AC while both markers had no prognostic impact. Interestingly, tumor immune response experienced a controversial prognostic role in PDAC and AC. The role of the immune response

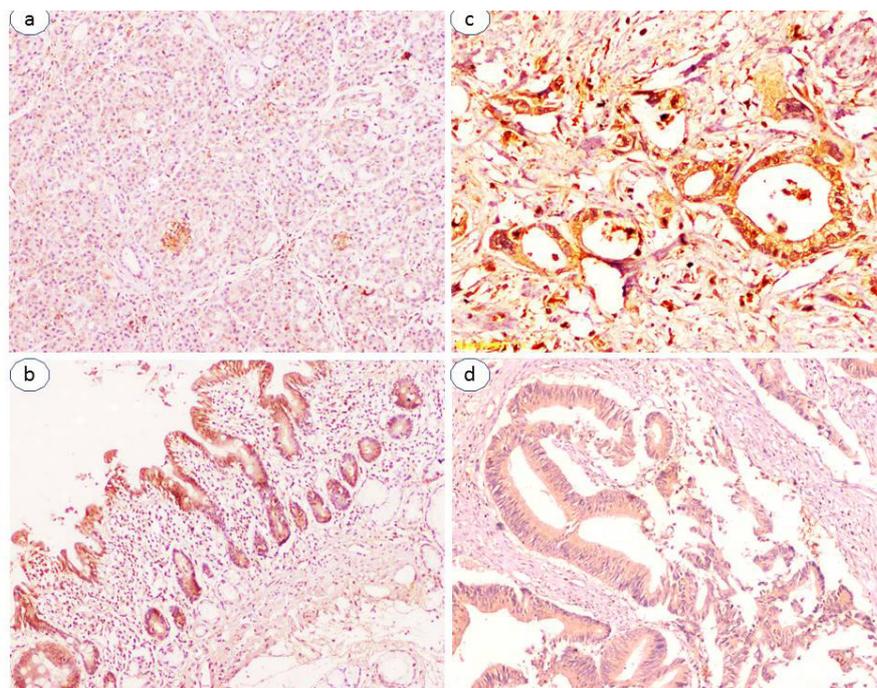


Figure 2. The Immunohistochemical Expression of A2AR in PDAC and AC. a) Negative expression of A2AR in adjacent non-tumor pancreatic duct (x100), b) Strong expression of A2AR in high grade PDAC (x200), c) Low expression of A2AR in adjacent non-tumor intestinal tissue (x100) and d) Low expression of A2AR in AC (x200).

in PDAC was modulated by CD73 and A2AR while no significant association between CD73/A2AR and AC immune response was observed.

In the current study, PDAC cases showed a significantly larger tumor size, positive perineural invasion, and involved surgical margin than AC. This is agreed with Sommerville et al., who reported that PDAC is usually diagnosed at a late onset which adversely impacted patients' prognosis and survival (Sommerville et al., 2009). However, there was no significant difference between both groups regarding the survival data which could be explained by the limited number of cases. Alternatively, Smeenk et al. reported that PDAC had the worst OS even after adjustment of tumor size, lymph node metastasis, and stage (Smeenk et al., 2007). Although, PDAC and AC shared a common embryological derivation and close origin proximity, they may be activated through distinct molecular pathways which could impact their clinical behavior (Ando, 2010; Sikdar et al., 2018). Our study supported this hypothesis and found a distinct role of CD73 and A2AR in the development and progression of PDAC and AC.

This study showed a significant overexpression of CD73 in PDAC, and AC compared to their adjacent non-tumor tissues. However, A2AR was overexpressed in PDAC only with no significant difference in its expression in the AC group. A possible explanation is that PAAC subgroups shared similar common carcinogenic pathways, whereas certain gene alterations are unique for some PAAC subtypes (Sikdar et al., 2018). This is consistent with our findings and could be attributed to the difference in the pathogenic pathways regulating CD73/A2AR expression in either group. The combinational overexpression of CD73 and A2AR in PDAC may indicate that CD73 mediated PDAC progression through induction of adenosine production (Messouadi et al., 2018; Katsuta and Takabe, 2019). The adenosinergic pathway further activates the secretion and expression of TGF- β , which in turn mediates tumor progression and the activation of epithelial-mesenchymal transition (EMT) (Garcia-Rocha et al., 2018). However, in AC, CD73 could enhance tumor growth independent of adenosine through activating several transcription factors such as PPAR γ and EGFR (Zhi et al., 2012; Zhu et al., 2017).

We hypothesized that the non-significant difference of A2AR expression in AC and non-tumor tissue could be attributed to two factors. First, CD73 induced A2AR expression may require the co-operation of other enzymes; nicotinamide adenine dinucleotide (NAD⁺), the alkaline phosphatase (ALP), and the prostatic acid phosphatase (PAP) which may be devoid in AC (Zimmermann et al., 2012). The other factor could be the early activation of A2AR in non-tumor intestinal tissue as a part of its physiological inflammatory role (Odashima et al., 2005). Therefore, further studies are recommended to clarify the exact mechanism of CD73 and A2AR activation and their potential role in the development of PDAC and AC.

The present study demonstrated a variable prognostic impact of CD73 and A2AR in PAAC groups. CD73 and A2AR showed an opposing effect on the PDAC

prognosis whereas both proteins have no relevant role in AC prognosis. CD73 overexpression was the most independent prognostic factor associated with the long-term OS of PDAC. This is agreed with the previous studies that reported the good prognostic impact of CD73 on breast, ovarian and urothelial cancers (Supernat et al., 2012; Wettstein et al., 2015). They linked its favorable role to the production of extracellular adenosine that inhibits cellular proliferation and induces tumor apoptosis in cell lines and human cancers (El-Darahali et al., 2005). On the contrary, other studies reported the poor prognostic impact of CD73 in PDAC (Katsuta and Takabe, 2019; Zhou et al., 2019). The conflicting results of the prognostic impact of CD73 in different human cancers could result from different etiological factors, molecular tumor subtyping, hormonal modulation, and the activation of different downward regulatory proteins (Gao et al., 2014). Regarding the prognostic role of A2AR in PDAC cases, this study showed a significant association between A2AR overexpression and high pathological grade and late tumor stage. This agreed with Wu et al., (2019) who found that higher expression levels of A2AR were significantly associated with larger tumor size, more tumor invasion, and advanced stage in colorectal carcinoma. This could be attributed to the role of A2AR in inducing EMT, promoting tumor cell proliferation, and functionally and metabolically suppressing tumor-infiltrating T lymphocytes, resulting in tumor immune evasion and progression (Mastelic-Gavillet et al., 2019). We postulated that CD73 is expressed in early tumor development; then its expression declines and is replaced by A2AR in advanced tumors. The second explanation is that CD73 and A2AR are regulated by complex signaling pathways that impact their function in a tissue-dependent manner. The opposing function of CD73 and A2AR in the current study was in context with Inoue et al., (2017b) who reported a similar result in lung carcinoma. However, in Inoue et al.'s study, CD73 showed a poor prognostic impact in contrast to the good prognostic impact of A2AR. Moreover, both markers showed no prognostic impact on AC prognosis. Therefore, further studies are recommended to elucidate the prognostic impact of CD73 and A2AR in PAAC.

Finally, we studied the role of immune response "TIMC" on the prognosis of PDAC and AC and how they impacted the function of CD73 and A2AR. Not surprisingly, TIMC exerted an opposed effect on both groups in the current study. Rich TIMC was significantly observed in PDAC cases of large tumor size and was correlated with low CD73 and high A2AR expressions. This agreed with Thike et al., (2020) who reported the role of high TIMC density in predicting breast carcinoma recurrence. This is agreed with Sciarra et al., (2019) found that CD73-positive cases showed low TIMC levels in hepatobiliary pancreatic tumor. In the current study, TIMC in PDAC could contribute to tumor aggressiveness through modulating CD73 and A2AR expressions.

The crosstalk between CD73/A2AR and tumor immune response is not well elucidated. In PDAC, CD73 was upregulated in the initial PDAC stage (small tumor size) and was associated with low TIMC. CD73 could

participate in immune suppression through mediating adenosine production to modulate the T-cell function and inducing the production of different angiogenic and tolerogenic factors, which inhibit the immune response (Jin et al., 2010; Ryzhov et al., 2011). However, the poor prognostic role of A2AR and its strong association with high TIMC in PDAC could be mediated by CD39 activation and hypoxia-induced factor independent of CD73 (Vigano et al., 2019). Also, the prognostic role of tumor immune response differs according to the type of tumor immune cells, the CD8/CD3 versus FoxP3+ lymphocytes, which are mediated by adenosine (Liang et al., 2018; Vigano et al., 2019; Orhan et al., 2020). Considering this, A2AR activation either has an immunosuppressive or immune-activating role according to the predominant immune cells in the tumor microenvironment and the downregulating pathway.

On the other hand, high TIMC levels were associated with a good prognosis in the AC group. The good prognostic impact of TIMC on AC could be attributed to several factors. First, the immune-modulatory effect of TIMC in eliminating neoplastic cells by activating T-cells and antigen-presenting cells, hence allowing disease control (Kim et al., 2019; Tagliabue et al., 2020). Second, the immuno-inflammatory cells could suppress the host antitumor immunity and prevent tumor migration and invasion (Calik et al., 2019). Lastly, the role of TIMC in AC was independent of CD73/A2AR activation since there was a lack of association of TIMC with either CD73 or A2AR expression. However, further studies are recommended to understand the crosstalk between TIMC and CD73/A2AR pathway in PAAC and whether its function is organ-specific or not.

The current study indicated that PDAC and AC differed at several levels including clinicopathological, survival parameters, CD73/A2AR function and, the impact of tumor immune response. CD73 and A2AR experience an opposed function on PDAC which is strongly correlated with the tumor immune response. CD73 was associated with long survival. Alternatively, A2AR was associated with the more aggressive PDAC cases. However, both markers had a limited role in AC and did not impact the prognostic role of tumor immune response.

Author Contribution Statement

All authors of this paper have participated in its drafting and approved the final version submitted. DS and AS wrote the paper, revised, and edited the final version. MT participated in clinical data collection and contributed to data interpretation and analysis. SA contributed to the pathological specimen retrieval, data collection and interpretation.

Acknowledgements

Not applicable.

Ethical approved

This study was approved by Institutional Review

Board (IRB) of Menoufia University

Availability of data

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors of this study have no conflicts of interest to declare.

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