Evaluation of the expression and role of adenosine receptor and programmed death ligand-1 in bladder urothelial carcinoma

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Received: 28 May 2021
Revised: 22 June 2021
Accepted: 16 July 2021
Published: 16 February 2022

Egyptian Journal of Pathology 2021, 41:65–72

Background
Immune-checkpoint molecules are important regulators of physiologic inflammatory responses, and are highly effective in behavior of many cancers. They are involved in production of antitumor immune response. This study aims to evaluate the expression and role of both programmed death ligand-1 (PDL-1) and adenosine receptor (A2aR) antibodies in bladder urothelial cancer.
Materials and methods
This was a retrospective study on 45 bladder urothelial cancers obtained from archives of Pathology Department, Faculty of Medicine, Benha University and from International Medical Center Hospital (IMC) during the period from January 2015 to December 2019. Immunohistochemical expression of A2aR was evaluated and compared with those of PDL-1. A2aR expression was also correlated with tumor-infiltrating CD8+T cells. Relations to demographic data of the patients were evaluated.
Results
PDL-1 expression was detected in 24.4% of tumor cells±immune cells. PDL-1 was significantly related to advanced PT stage ($P<0.01$), detrusor muscle invasion ($P<0.05$), and nonpapillary tumor histology ($P< 0.05$). High expression of A2aR was detected in 40% of tumor specimens in tumor cells±immune cells. Higher A2aR expression was statistically significant related to higher tumor grade ($P<0.05$), advanced PT stage ($P<0.01$), detrusor muscle invasion ($P<0.01$), and nonpapillary tumor histology ($P< 0.01$). The expression of A2aR was statistically significant related to PDL-1 expression ($P< 0.01$). Higher A2aR expression was statistically related to lower the density of tumor-infiltrating CD8+T lymphocytes ($P< 0.01$). Using receiver operating characteristic curve, A2aR was more accurate than PDL-1 as area under the curve (0.648 and 0.565, respectively). The specificity of A2aR is higher than PDL-1 (51.9 and 29.6, respectively).
Conclusion
Both PDL-1 and A2aR markers could be useful in monitoring urothelial bladder cancer immunotherapy. A2aR is higher to and more specific than PDL-1 in predicting tumor lymphocytic infiltrate. A2aR antagonists could have a positive role in cases that are resistant to anti-PDL-1 immunotherapy.

Keywords:
adenosine receptor, bladder urothelial carcinoma, immunotherapy, programmed death ligand-1

Egypt J Pathol 41:65–72

1687-4277

Introduction

In spite of intensive treatment programs, bladder urothelial cancer (BUC) remains a major health problem in Egypt. It is the third most common cancer corresponding to 7% of total malignancies (Helal *et al*., 2015). According to American Cancer Society 2019, bladder cancers are classified into two subtypes: non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC), depending on microscopic findings (Smith *et al*., 2014).

Clinically, both types are mainly managed surgically. However, despite progress in surgical techniques, the prognosis of BUC patients after surgical treatment is still poor. One-third of NMIBC patients would suffer from cancer relapse and progress, while the 5-year survival for MIBC is only 50–60% (Kaufman *et al*., 2009). Moreover, the response to radiotherapeutic or chemotherapeutic treatment for patients with advanced BUC remains unsatisfactory. Thus, it is valuable to study the underlying molecular mechanisms and trying to identify new helpful biomarkers that could be used to improve the

outcome of cancer and control its metastatic potential (Parekh *et al.*, 2006).

Multiple mechanisms in tumor microenvironment are responsible for impairment of the control of cytotoxic T-cell influence on tumor growth. For example, when tumor-rejection antigens are poorly expressed on tumor cells, adenosine molecules could prevent tumor cell killing by activation of adenosine receptor (A2AR) signaling (Mastelic-Gavillet *et al.*, 2019).Under hypoxic conditions, adenosine is generated by decomposition of ATP through the action of ectonucleotidases CD73 and CD39. It is a critical immunosuppressive molecule in tumor microenvironment (Young *et al.*, 2018). Hypoxic conditions like inflammation, trauma, ischemia, and anoxia lead to increase of extracellular adenosine concentrations about hundred times in comparison with normal levels (Estrela and Abraham 2011). Four subtypes of ARs are recognized: A1, A2a, A2b, and A3 (Hasko *et al.*, 2008). Multiple studies found that A2b receptor (A2bR) was the highly expressed in different types of cancers due to the hypoxic microenvironments (Mousavi *et al.*, 2015). Suppression of A2bR expression was related to inhibition of cell proliferation in oral cancer (Kasama *et al.*, 2015). Also, the A2bR antagonist resulted in slow rate of growth of 4T1 breast tumor cells (Cekic *et al.*, 2012). These findings, together, suggest that A2bR may have an oncogenic function. To the best of our knowledge, the pattern of expression and biological function of A2aR (also known as ADORA2a) in BUC have not been examined.

In our previous study on programmed death ligand-1 (PDL-1) in BUC, we concluded that PDL-1 may be used as a prognostic marker for tumor aggressiveness and, it could be involved in tumor immunotherapy (El Sawi *et al.*, 2020). Anti-PDL-1 antibodies were later approved for treatment of many cancers. Unfortunately, because of its resistance, only a limited proportion of patients could benefit. The mechanisms controlling anti-PDL-1 resistance are not understood. Overcoming this resistance is still under trials (Fong *et al.*, 2020).

Stimulation of A2aR antigen on the surface of tumor-immune cells will inhibit antitumor activities of these immune cells, therefore, paving the way for immunosuppressive tumor microenvironment (Inoue *et al.*, 2017). As concluded in the study done by Kamai *et al.* (2021), the expression of A2AR and PDL-1 in the primary renal cell carcinomas could predict the treatment outcomes on using anti-VEGF agents and that the A2aR antagonists may be effective molecular targets for immunotherapy.

This study aimed to assess the possible role of A2aR in urothelial bladder carcinoma and to determine the relation between its expression and different clinicopathological findings. Also, to compare the A2aR results with those of PDL-1 previously obtained trying to predict their role in assessment of BUC behavior and management.

Materials and methods

Study groups

This retrospective study included selected 45 cases of urothelial bladder carcinomas further divided into 21 muscle-invasive BUCs and 24 non-muscle-invasive urothelial carcinomas. Included six cases of persistent dysuria for unknown cause were considered as controls. Specimens were obtained by radical cystectomy (12 cases) and by transurethral resection of urinary bladder (33 cases). Studied cases were selected according to available clinicopathological data and available wax blocks. Patients' relevant demographic data were retrieved from the medical records of patients.

Histopathological studies

Paraffin-embedded specimens were obtained from archives of Pathology Departments, Faculty of Medicine, Benha University and International Medical Center Hospital (IMC). Cases were collected during the period from January 2015 to December 2019. Hematoxylin and eosin sections were reviewed by two independent pathologists to confirm diagnosis. The histopathological type was reviewed and graded according to WHO classification, 2016 (Compérat *et al.*, 2019), and staged according to American Joint Committee on Cancer (AJCC) Staging System, 8th edition (Wang and McKenney, 2019). The density of lymphocytic infiltrate was reported according to the International TILs Working Group (ITWG) 2014 (Hendry *et al.*, 2017; Fuchs *et al.*, 2020). Ethical approvals from Research Ethics Committee of Faculty of Medicine, Benha University, and International Medical Center Hospital were obtained.

Immunohistochemistry

For immunohistochemical staining, 4-mm sections were cut from paraffin blocks and placed on positive-charged slides. For A2aR, the primary antibodies used were mouse monoclonal adenosine A2AR (7F6-G5-A2) antibody (Cat.# sc-32261; Santa Cruz Biotechnology, Dallas/Texas, USA) at a dilution of 1 : 100 and *CD8* rabbit monoclonal antibody (Cat. # MA5-16345; Thermo Fisher Scientific Anatomical Pathology, USA) at a dilution of 1 : 100. The detection kit was Ultravision detection system (Cat#, TP-015-HD, Lab

Vision, USA). Antigen retrieval was done by using 10 mmol/l citrate monohydrate buffer (pH 6.0) and heating for 15 min in the microwave. 3,3′-Diaminobenzidine tetrahydrochloride (DAB) was used as chromogen. Positive control for CD8 was tonsillar tissue and for A2aR was intratumoral immune cells showing positive staining. Negative-control slides were obtained by using cold phosphate-buffered saline instead of primary antibody.

Previously GATA3 and CK 5/6 antibody-stained slides were obtained from the patient archives and reassessed for molecular classification of studied cases. The results of PDL-1 staining obtained in our previous study (El Sawi *et al.*, 2020) were recruited for a comparative study of the roles of PDL-1 and A2aR in BUC.

Interpretation of immunohistochemical results

The A2aR was detected as brown cytomembranous staining in both tumor cells and tumor-infiltrating immune cells. The level of A2aR expression was scored (0–3). Low expression was considered if less than 5% of both tumor cells and immune cells were positive (score 0–1). High expression was considered if more than 5% of either tumor cells or immune cells or both were positive (score 2–3), irrespective of staining intensity (Kamai *et al.*, 2021).

Statistical analysis

Data of thesis were analyzed using SPSS, version 20 software (SPSS Inc., Chicago, Illinois, USA). The association between markers of the study and case groups was done using Pearson correlation coefficient. The accepted level of significance was 0.05 ($P<0.05$ was considered significant, $P<0.01$ was considered as highly significant). Receiver operating characteristic (ROC) curve was made for the validity of A2aR and PDL-1 in prediction of the density of tumor-infiltrating CD8+ T cells.

Results

Included in this study were 45 urothelial carcinomas. Among the studied cases, 36 (72%) were males and nine (18%) were females. The age of patients ranged from 31 to 78 years with mean age 61.11. Studied cases were classified as 24 (53.3%) cases of NMIBC and 21 (46.7%) cases of MIBC. Grading of studied cases

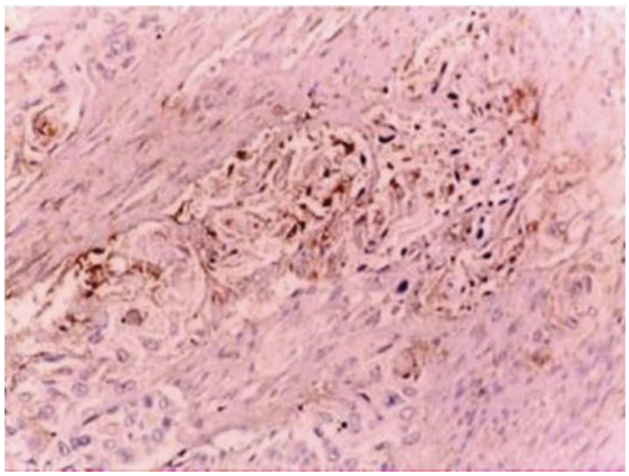
Table 1 Relations between clinicopathological data of studied bladder urothelial carcinoma cases and the expression of both adenosine receptor and programmed death ligand-1

Variables	Total	A2aR [*n* (%)]		*P* value	PDL-1 [*n* (%)]		*P* value
		Low expression	High expression		Negative	Positive	
Tumor size (cm)							
< 3	21	15 (71.4)	6 (28.6)	>0.05	17 (81)	4 (19)	>0.05
≥3	24	12 (50)	12 (50)		17 (70.8)	7 (29.2)	
Muscle invasion							
NMIBC	24	20 (83.3)	4 (16.7)	<0.01*[*]	21 (87.5)	3 (12.5)	<0.05[*]
MIBC	21	7 (33.3)	14 (66.7)		13 (61.9)	8 (38.1)	
Grade							
Low grade	18	14 (77.8)	4 (22.2)	<0.05[*]	16 (88.9)	2 (11.1)	>0.05
High grade	27	13 (48.1)	14 (51.9)		18 (66.7)	9 (33.3)	
Histological variant							
Papillary	25	20 (80)	5 (20)	<0.01[**]	22 (88)	3 (12)	<0.05[*]
Nonpapillary	20	7 (35)	13 (65)		12 (60)	8 (40)	
T stage							
Ta	12	11 (91.7)	1 (8.3)		11 (91.7)	1 (8.3)	
T1	12	9 (75)	3 (25)	<0.01[**]	10 (83.3)	2 (16.7)	<0.01[**]
T2	16	6 (37.5)	10 (62.5)		13 (81.2)	3 (18.8)	
T3	3	1 (33.3)	2 (66.7)		0	3 (100)	
T4	2	0	2 (100)		0	2 (100)	
LVI							
Positive	7	3 (42.9)	4 (57.1)	>0.05	5 (71.4)	2 (28.6)	>0.05
Negative	38	24 (63.2)	14 (36.8)		29 (76.3)	9 (23.7)	
Molecular subtype							
Luminal	25	21 (84)	4 (16)		23 (92)	2 (8)	>0.05
Basal	14	4 (28.6)	10 (71.4)	>0.05	7 (50)	7 (50)	
Double negative	6	2 (33.3)	4 (66.7)		4 (66.7)	2 (33.3)	
Total	45	27 (60)	18 (40)		34 (75.6)	11 (24.4)	

A2aR, adenosine receptor; MIBC, muscle invasive bladder cancer; NMIBC, non-muscle invasive bladder cancer; PDL-1, programmed death ligand-1. [*]Significant data. [**]Highly significant data.

revealed 18 (40%) cases of low grade and 27 (60%) of high-grade urothelial cancers. Histologically, this study included 25 (55.6%) cases of papillary urothelial carcinoma and 20 (44.4%) cases of nonpapillary patterns (nested, solid, and micropapillary). Regarding T stage, 12 (26.7%) cases of PTa, 12 (26.7%) cases of PT1, 16 (35.6%) cases of PT2, three (6.6%) cases of PT3, and two (4.4%) cases of PT4. On evaluation of lymphovascular invasion, negative cases were 38 (84.4%) and positive cases were seven (15.6%). Previous staining results of CK 5/6 and GATA3 antibodies revealed 25 (55.6%) luminal-type cases, 14 (31.1%) basal-type cases, and six (13.3%) double-negative cases.

Figure 1



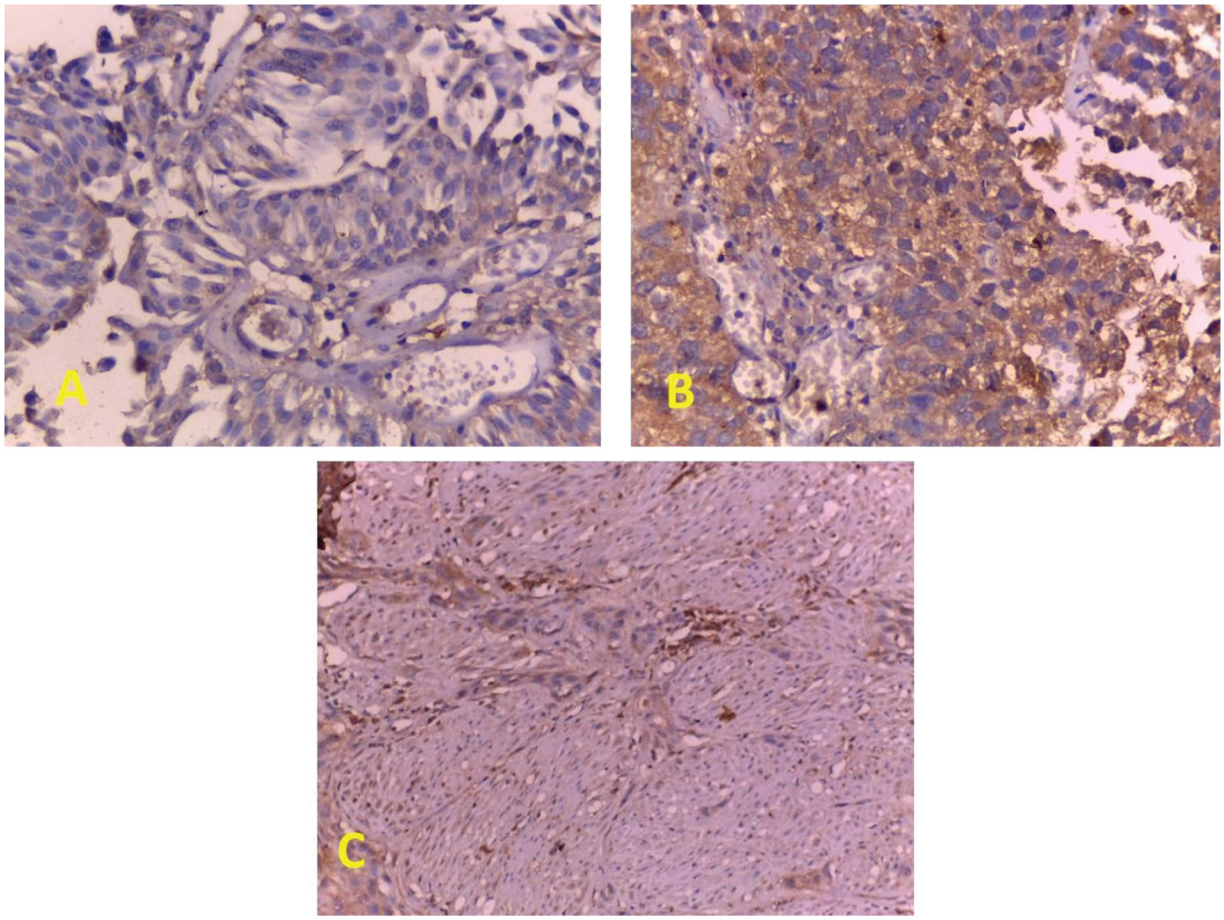
Positive cytoplasmic PDL-1 expression in tumor groups of cells invading muscularis propria (IHC ×400). IHC, immunohistochemistry; PDL-1, programmed death ligand-1.

Immunohistochemical results

Our previous study on PDL-1 revealed that it was positively expressed in 24.4% of tumor cells±immune cells. PDL-1 was significantly related to advanced PT stage (*P*?0.01), detrusor muscle invasion (*P*?0.05), and nonpapillary tumor histology (*P*? 0.05) (Table 1) (Fig. 1).

Positive A2aR expression was detected as brown cytoplasmic and/or membranous staining of both tumor cells and immune cells infiltrating the tumor (Fig. 2). High expression was detected in 40% of tumor

Figure 2



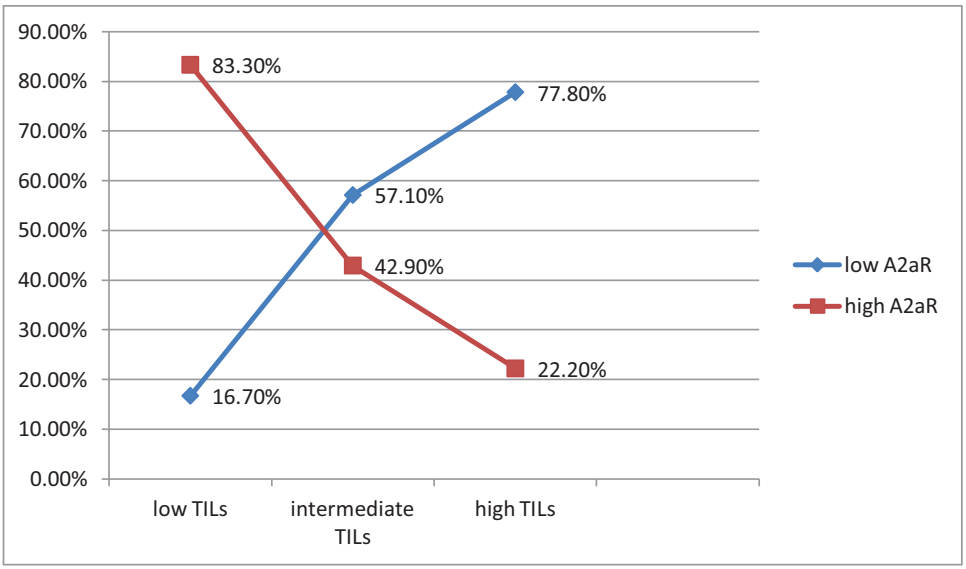
(a) Low cytoplasmic A2aR expression in papillary low-grade urothelial carcinoma (IHC ×400). (b) High cytoplasmic A2aR expression in solid high-grade urothelial carcinoma (IHC ×400). (c) High cytoplasmic A2aR expression in tumor groups of cells invading muscles (IHC ×200). A2aR, adenosine receptor; IHC, immunohistochemistry.

Table 2 Relation of adenosine receptor expression to programmed death ligand-1 expression

	Total (*N*=45)	Low expression A2aR [*n* (%)]	High expression A2aR [*n* (%)]	*P* value
Negative PDL-1	34	25 (73.5)	9 (26.5)	<0.01
Positive PDL-1	11	2 (18.2)	9 (81.8)	

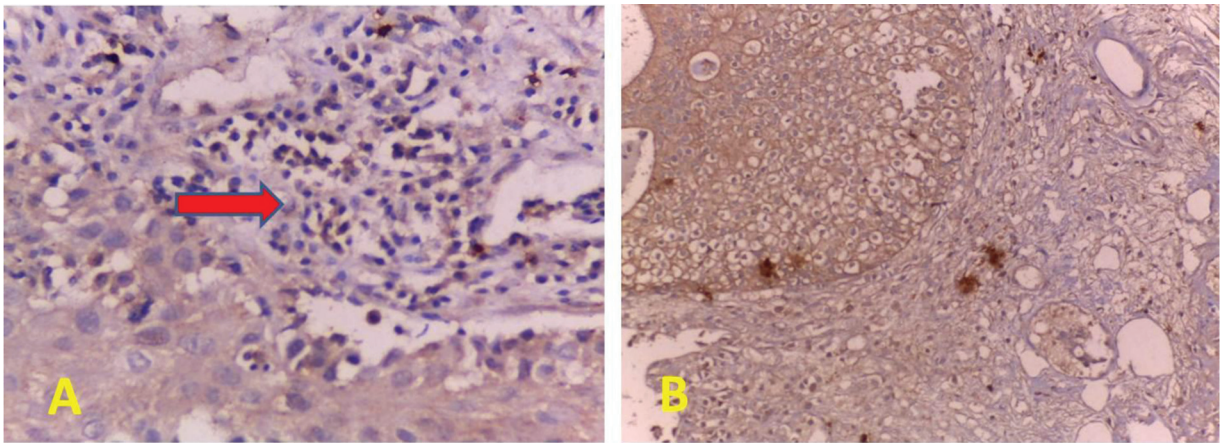
A2aR, adenosine receptor; PDL-1, programmed death ligand-1.

Graph 1



Relation of A2aR expression to the density of tumor-infiltrating CD8+T cells. A2aR, adenosine receptor.

Figure 3



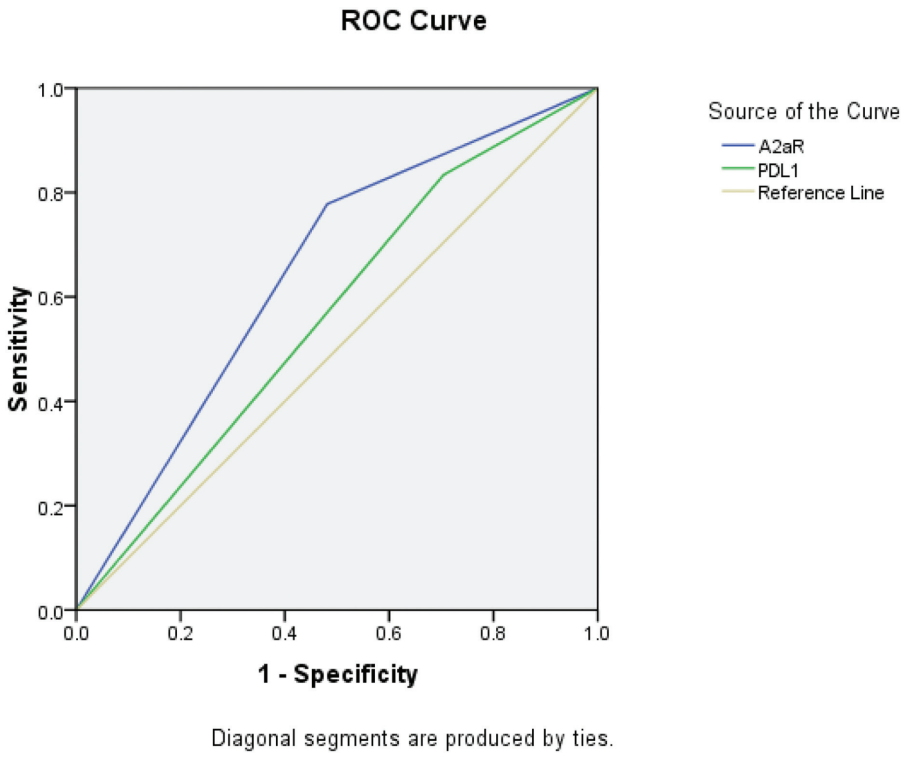
(a) Positive CD8 expression of tumor-infiltrating T cells (red arrow) (IHC ×400). (b) High A2aR cyto-/membranous expression with low CD8+T-cell density (IHC ×200). A2aR, adenosine receptor; IHC, immunohistochemistry.

specimens in tumor cells±immune cells. Higher A2aR expression was statistically significant related to higher tumor grade (*P*? 0.05) (Fig. 2a, b), advanced PT stage (*P*?0.01), detrusor muscle invasion (*P*? 0.01) (Fig. 2c), and nonpapillary tumor histology (*P*? 0.01) (Table 1) (Fig. 2a, b). The expression of A2aR was statistically significant related to PDL-1 expression. Among positive PDL-1 cases, 81.8% showed high expression of A2aR (*P*? 0.01) (Table 2).

Additionally, in the examined cases, it was noted that the higher A2aR expression, the lower the density of tumor-infiltrating CD8+T lymphocytes, a high statistically significant inverse relation (*P*? 0.01) (Graph 1) (Fig. 3).

ROC curve was made for the validity of A2aR and PDL-1 in prediction of the density of TILs. A2aR is more accurate than PDL-1 as area under the curve (0.648 and 0.565, respectively). Specificity of A2aR is

Graph 2



ROC curve for the validity of A2aR and PDL-1 in prediction of the density of TILs. A2aR, adenosine receptor; PDL-1, programmed death ligand-1; ROC, receiver operating characteristic.

Table 3 Validity of adenosine receptor and programmed death ligand-1 in prediction of density of TILs

	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
A2aR	0.648	77.8	51.9	51.9	77.8	62.2
PDL-1	0.565	83.3	29.6	44.1	72.7	51.1

A2aR, adenosine receptor; AUC, area under the curve; NPV, negative predictive value; PDL-1, programmed death ligand-1; PPV, positive predictive value.

higher than PDL-1 (51.9 and 29.6, respectively) (Graph 2 and Table 3).

Discussion

Regulation of the duration and strength of immune response is a critical issue of immunity. Multiple mechanisms are working for protection of normal tissue from autoimmunity or an exaggerated reaction to pathogens. Inhibitory signals, or 'immune check points,' are upregulated on surfaces of activated lymphocytes. While checkpoint pathways are crucial for controlling physiologic inflammatory reactions, also, they are highly active in many cancers and are considered an important mechanism to escape the antitumor immune response (Leone and Emens, 2018).

A2AR, the main functional AR on T cells, is playing a strong role in the limitation of inflammation and tissue damage (Liang *et al*., 2014). Tissue hypoxia is an essential factor for inducing adenosine-generation pathways CD73 and CD39. Consequently, ARs as A2AR are activated due to increased extracellular adenosine levels in tumor microenvironment (Ma *et al*., 2017).

In this study on BUCs, high A2aR expression was statistically significantly related to higher tumor grade ($P< 0.05$). These results agreed with Ma *et al*. (2017) who reported in their study on squamous-cell carcinoma that overexpression of 2AR was related to tumor pathological grade. However, Guimei and Eladl (2021) in their work on breast carcinoma and Wu *et al*. (2019) in their work on colorectal cancer, reported an insignificant relation between A2aR expression and grade of tumor. This may be attributed to different types of tissue studied and different grading systems.

Our results revealed a high statistically significant relation of A2aR expression with advanced PT stage ($P<0.01$) and detrusor muscle invasion ($P< 0.01$). This agreed with the study by Wu *et al*. (2019) on

colorectal cancer. They reported that A2aR expression was linked to depth of tumor invasion (P=0.011) and TNM stage (P=0.005). Another statistical significant relation was found in this work between A2aR expression and nonpapillary tumor histology ($P<$ 0.01). This also could be explained by the tendency of nonpapillary urothelial tumors (solid, nested, and micropapillary subtypes included in this study) to invade the muscularis propria.

In their work on rhabdomyosarcoma, Tarnowski *et al.* (2020), suggested that adenosine and AMP stimulate cell migration and directing of cancer cells to sites of hypoxia or cellular damage; thus, adenosine may be involved in mechanisms controlling cancer cell metastasis. Extracellular adenosine was proved to induce chemotaxis of endothelial progenitors, neutrophils, and cancer cells. At the invasive tumor edge, migrating cancer cells with overexpression of ARs respond to external chemoattractant and thus increasing its migratory capacity.

Insignificant statistical relation of A2aR expression and tumor size ($P>$ 0.05) was found in this work. This agreed with Guimei and Eladl (2021). But it was against the results of Ma *et al.* (2017) and Wu *et al.* (2019). This difference may be attributed to the type of biopsy evaluated, which was not clear in both studies of Ma *et al.* (2017) and Guimei and Eladl (2021), while Wu *et al.* (2019) used only total excessional biopsies.

CD8+T-cell functions and metabolic influences are markedly impaired in tumor microenvironment. Extracellular adenosine, acting through the A2aR, is an effective negative regulator of CD8+T-cell development and function (Chen *et al.*, 2020).

In this study, dense CD8+T-cell infiltration was significantly associated with low A2aR ($P<$ 0.01). These results agreed with Ma *et al.* (2017), who worked on head and neck squamous cell carcinoma. They reported that A2aR was negatively related to CD8 expression and so, the authors considered that activation of A2aR may be linked to immunosuppression status in these tumors. Yu *et al.* (2016), in their experimental study reported that A2aR may have an immune-regulatory function by affecting CD8+T cells in 2cKO tumor-bearing mice. In the study by Ma *et al.* (2017), increased antitumor response of CD8+T cells was noted in 2cKO tumor-bearing mice that were treated by A2AR antagonist, and hence, improvement of the immunosuppressive tumor microenvironment occurred.

Mastelic-Gavillet *et al.* (2019) reported that adenosine is known to inhibit tumor immunity by decreasing immune cell infiltration, cytotoxicity, and production of cytokines. In a study by Longhi *et al.* (2013), A2aR activation resulted in reducing T-cell proliferation together with induction of T-cell apoptosis. So, A2AR was claimed to protect tumor cells from the antitumor CD8+T-cell effects.

According to Hendry *et al.* (2017), upregulation of immune-checkpoint molecules is related to activation of T cells and depends on their existence in the tumor microenvironment. So, tumor-infiltrating lymphocyte assessment alone could be valuable in predicting tumor cell response to inhibition of immune check points.

However, Guimei and Eladl (2021) reported in their work on breast tissue that A2aR expression was not related to the percentage of tumor-infiltrating lymphocytes. This confliction with our results may be due to different methods used in assessment density of tumor-infiltrating lymphocytes.

In our previous work on BUC, PDL-1 was positively expressed in 24.4% of tumor cells±immune cells. PDL-1 was significantly related to PT stage ($P<$0.01), muscle-invasive pattern ($P<$ 0.05), and nonpapillary (solid, nested, and micropapillary) variants ($P<$ 0.05). Conversely, PDL-1 was not related to tumor grade ($P>$ 0.05). PDL-1 was also not related to tumor size, lymphovascular invasion, or molecular subtype.

In this work, assessment of A2aR expression was done in the same tissue specimens evaluated before, for PDL-1. Forty percent of tumor specimens examined were positive for A2aR expression. Additionally, its expression was highly statistically related to PDL-1 expression ($P<$ 0.01). This relation between the expression of two markers agreed with the results of Ma *et al.* (2017). They reported a positive association between the expression of both markers in colorectal cancer ($P<$ 0.01).

Several studies concluded that PDL-1 is expressed by antigen-presenting cells and is thought to inhibit T lymphocytes. PDL-1 can cause downregulation of proliferating antigen-stimulated lymphocytes and decrease cytokine production by binding to its specific receptor (PD-1), which consequently leads to immune tolerance (Chen *et al.*, 2016).

In the immune microenvironment, combination of adenosine to A2aR activates production of cAMP.

Then cAMP can suppress the immunological response of the human body through initiating other costimulatory molecules in a multistep process. The whole process can inhibit the antitumor immune response of T cells, and improve proliferation of immunosuppressive cells, thus permitting the tumor to get immune escape (Wu *et al*., 2019). Studies on animal models have revealed that prior treatment with anti-PD-1 antibodies resulted in increased expression of A2AR and CD73, suggesting that the adenosine pathway may result in therapeutic resistance to immunotherapy (Beavis *et al*., 2015). To the best of our knowledge, no authors before used ROC curve to study the relation between A2aR and PDL-1 expression regarding tumor lymphocytic infiltrate. According to the displayed ROC curve, A2aR could be considered more accurate than PDL-1 as area under the curve (0.648 and 0.565, respectively). Specificity of A2aR is higher than PDL-1 (51.9 and 29.6, respectively).

Conclusion

Both PDL-1 and A2aR markers could be useful in monitoring urothelial bladder cancer immunotherapy. A2aR is higher to and more specific than PDL-1 in predicting tumor lymphocytic infiltrate. A2aR antagonists could have a positive role in cases that are resistant to anti-PDL-1 immunotherapy.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

Beavis PA, Milenkovski N, Henderson MA, John LB, Allard B, Loi S, *et al.* (2015). Adenosine receptor 2A blockade increases the efficacy of anti-PD-1 through enhanced antitumor T-cell responses. Cancer Immunol Res 3:506–517.

Cekic C, Sag D, Li Y, Theodorescu D, Strieter RM, Linden J. (2012). Adenosine A2B receptor blockade slows growth of bladder and breast tumors. J Immunol 188:198–205.

Chen J, Jiang CC, Jin L, Zhang XD. (2016). Regulation of PDL1: a novel role of pro-survival signalling in cancer. Ann Oncol 27:409–416.

Chen S, Akdemir I, Fan J, Linden J, Zhang B, Cekic C. (2020). The expression of adenosine A2B receptor on antigen-presenting cells suppresses CD8? T-cell responses and promotes tumor growth. Cancer Immunol Res 8:1064–1074.

Compérat EM, Burger M, Gontero P, Mostafid AH, Palou J, Rouprêt M, *et al.* (2019). Grading of urothelial carcinoma and the new 'World Health Organisation Classification of Tumours of the Urinary System and Male Genital Organs 2016'. European Urology Focus (EUF) 5:457–466.

El Sawi RM, Abdel Aal E, El-Sebaaie A, Zafer N, Agina H (2020). Significance of expression of IMP3, PPARG & PD-L1 in urothelial carcinoma (immunohistochemical study). Benha Med J 38:112–127.

Estrela AB, Abraham WR (2011). Adenosine in the inflamed gut: a janus faced compound. Curr Med Chem 18:2791–2815.

Fong L, Hotson A, Powderly JD, Sznol M, Heist RS, Choueiri TK, *et al.* (2020). Adenosine 2A receptor blockade as an immunotherapy for treatment-refractory renal cell cancer. Cancer Discov 10:40.

Fuchs TL, Sioson L, Sheen A, Jafari-Nejad K, Renaud CJ, Andrici A, *et al.* (2020). Assessment of tumor-infiltrating lymphocytes using International TILs Working Group (ITWG) system is a strong predictor of overall survival in colorectal carcinoma. A study of 1034 patients. Am J Surg Pathol 44:536–544.

Guimei M, Eladl MA (2021). Expression of adenosine receptors in young patients with breast cancer and its association with high proliferative index. IJCBR 5:57–66.

Hasko G, Linden J, Cronstein B, Pacher P (2008). Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. Nat Rev Drug Discov 7:759–770.

Helal TA, Salman MI, Ezz-Elarab SS (2015). Malignant tumors of the urinary bladder. In: Malignant urinary system tumors. Pathology-based cancer registry 2001-2010. Cairo, Egypt: Ain Shams Faculty of Medicine; Chapter 7, p. 63–72.

Hendry S, Salgado R, Gevaert T, Russell PA, John T, Thapa B, *et al.* (2017). Assessing tumor-infiltrating lymphocytes in solid tumors: a practical review for pathologists and proposal for a standardized method from the International Immuno-Oncology Biomarkers Working Group: part 2: TILs in melanoma, gastrointestinal tract carcinomas, non-small cell lung carcinoma and mesothelioma, endometrial and ovarian carcinomas, squamous cell carcinoma of the head and neck, genitourinary carcinomas, and primary brain tumours. Adv Anat Pathol 24:311–335.

Inoue Y, Yoshimura K, Kurabe N, Kahyo T, Kawase A, Tanahashi M, *et al.* (2017). Prognostic impact of CD73 and A2A adenosine receptor expression in non-small-cell lung cancer. Oncotarget 8:8738–8751.

Kamai T, Kijima T, Tsuzuki T, Nukui A, Abe H, Arai K, Yoshida K (2021). Increased expression of adenosine 2A receptors in metastatic renal cell carcinoma is associated with poorer response to anti?vascular endothelial growth factor agents and anti?PD?1/Anti?CTLA4 antibodies and shorter survival. Cancer Immunol Immunotherapy 70:2009–2021.

Kasama H, Sakamoto Y, Kasamatsu A, Okamoto A, Koyama T, Minakawa Y, *et al.* (2015). Adenosine A2b receptor promotes progression of human oral cancer. BMC Cancer 15:563.

Kaufman DS, Shipley WU, Feldman AS (2009). Bladder cancer. Lancet 374:239–249.

Leone RD, Emens LA (2018). Targeting adenosine for cancer Immunotherapy. ImmunoTherapy Cancer 6:57.

Liang D, Zuo A, Shao H, Chen M, Kaplan HJ, Sun D (2014). Roles of the adenosine receptor and CD73 in the regulatory effect of CD T cells. PLoS ONE 9:e108932.

Longhi MS, Robson SC, Bernstein SH, Serra S, Deaglio S(2013). Biological functions of ecto-enzymes in regulating extracellular adenosine levels in neoplastic and inflammatory disease states. J Mol Med 91:165–172.

Ma S, Deng W, Liu J, Mao L, Yu G, Bu L, *et al.* (2017). Blockade of adenosine A2A receptor enhances CD8+ T cells response and decreases regulatory T cells in head and neck squamous cell carcinoma. Mol Cancer 16:99.

Mastelic-Gavillet B, Rodrigo BN, Décombaz L, Wang H, Ercolano G, Ahmed R, *et al.* (2019). Adenosine mediates functional and metabolic suppression of peripheral and tumor-infiltrating CD8+ T cells. J Immunother Cancer 7:257.

Mousavi S, Panjehpour M, Izadpanahi MH, Aghaei M (2015). Expression of adenosine receptor subclasses in malignant and adjacent normal human prostate tissues. Prostate 75:735–747.

Parekh DJ, Bochner BH, Dalbagni G (2006). Superficial and muscle-invasive bladder cancer: principles of management for outcomes assessments. J Clin Oncol 24:5519–5527.

Smith AB, Deal AM, Woods ME, Wallen EM, Pruthi RS, Chen RC, *et al.* (2014). Muscle-invasive bladder cancer: evaluating treatment and survival in the National Cancer Data Base. BJU Int 114:719–726.

Tarnowski M, Tkacz M, Piotrowska K, Zgutka K, Pawlik A (2020). Differential effect of adenosine on rhabdomyosarcoma migration and proliferation. Arch Med Sci 16:414–427.

Wang G, McKenney JK (2019). Urinary bladder pathology: World Health Organization (WHO) classification and American Joint Committee on Cancer (AJCC) staging update. Arch Pathol Lab Med 143:571–577.

Wu Z, Yang L, Shi L, Song H, Shi P, Yang T, *et al.* (2019). Prognostic impact of adenosine receptor 2 (A2aR) and programmed cell death ligand 1 (PD-L1) expression in colorectal cancer. Biomed Res Int 2019:8014627.

Young A, Ngiow SF, Gao Y, Patch AM, Barkauskas DS, Messaoudene M, *et al.* (2018). A2AR adenosine signaling suppresses natural killer cell maturation in the tumor microenvironment. Cancer Res 78:1003–1016.

Yu GT, Bu LL, Zhao YY, Mao L, Deng WW, Wu TF, *et al.* (2016). CTLA4 blockade reduces immature myeloid cells in head and neck squamous cell carcinoma. Oncoimmunology 5:e1151594.