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It is with great pleasure that I write this editorial to welcome you to the IJCBR. This journal provides a platform for publication of original and reviews research articles, short communications, letter to editor, thesis abstract, conference report, and case studies. These types of publication are directed at the interface of the fields of cancer and biomedical research.

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I take this chance to welcome your contributions to the IJCBR and have every expectation that it will soon become one of the most respected journals in both the fields of cancer and biomedical research.

Mohamed L. Salem,

Editor in Chief

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (IJCBR)

RESEARCH ARTICLE

Expression of Adenosine Receptors in Young Patients with Breast Cancer and its Association with High Proliferative Index

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ABSTRACT

Background: Adenosine is produced in the hypoxic tumor milieu. By stimulating its receptors, it plays an important role not only in evading the body's immune mechanisms but also in enhancing tumor vasculature and contributing to tumor aggressiveness. **Aim:** The present study aimed at investigating the immunohistochemical expression of Adenosine receptors (A2A) in breast cancer tissue and its association with different clinico-pathological parameters. **Materials and Methods:** Thirty formalin-fixed paraffin-embedded (FFPE) breast cancer specimens were immunohistochemically stained using A2AR antibodies. All clinical and pathological data were retrospectively retrieved from the patients' records. **Results:** Cytoplasmic expression of A2ARs was noted not only on the tumor immune cells but also on the tumor cells themselves. They were strongly expressed on the tumor cells in 73.3% (n=22) of tumors. Expression was significantly higher in younger aged patients (< 50 years old) compared to older ones [p=0.044]. A2AR expression was also significantly higher in Luminal B and triple negative compared to Luminal A tumors [p=0.028]. The majority of A2AR expressing tumors had a mitotic index score of 2 [p=0.013] as well as a significantly higher proliferative index (ki-67 >20%) [p=0.018]. No association was observed between A2AR expression and tumor size, type, grade, Lymph node status, percentage of TILs or patient survival after 2 years of follow-up. **Conclusion:** These findings suggest a possible role for Adenosine in imparting a more aggressive phenotype in breast cancer and that targeting these receptors using Adenosine receptor antagonists could have a potential role in improving the outcome of these patients.

Keywords: Adenosine Receptors; Adenosine Receptors antagonists, luminal B, Triple negative, breast cancer.

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INTRODUCTION

Despite the great advances in Breast Cancer screening, early diagnosis, and treatment, it remains the most common cancer in females and the second most common cancer killer after lung cancer, with many cases still encountering therapeutic failures (Tarver, 2012). Therefore, the search for new prognostic and therapeutic molecules is still ongoing aiming to further improve the outcome and reduce the number of recurrences and metastasis in breast cancer patients.

In the past decade, there has been a considerable focus on the understanding of the complex interplay between the tumor cells and their surrounding microenvironment. Evasion of the anti-tumor immune response has emerged as one of the hallmarks of cancer as well as a key contributor to tumor progression and a widely sought-after therapeutic target. Numerous molecules are now recognized as targets for immunotherapy, also described as "immune checkpoints" (Allard et al., 2016; Inoue et al., 2017).

Currently, the clinical application of immune checkpoint targeting drugs has yielded great success in the treatment of many tumors. Antibodies against Programmed-death-ligand1 (PD-L1) and cytotoxic-T lymphocyte antigen-4 (CTLA-4) are currently FDA-approved drugs that are being successfully used for the treatment of

advanced malignant melanomas (Johnson et al., 2015). Amongst a newer generation of immuno-oncology drugs, those targeting adenosine-mediated immunosuppression via CD73 and Adenosine receptor (A2a) are currently being investigated (Allardet al., 2016; Antonioli et al., 2016).

Adenosine is an ancient signaling molecule that has a crucial role in regulating various cellular processes during cancer development. It is produced both intracellularly and extracellularly. Intracellular adenosine is produced from its immediate precursor, 5'-adenosine monophosphate (5'-AMP), by the action of the enzyme 5'-nucleotidase. This adenosine is important for energy and nucleic acid metabolism and can later be transported extracellularly (Sheth et al., 2014). Adenosine generated in the extracellular space is formed by the breakdown of ATP through a series of ectoenzymes, including (CD39) and (CD73) present on the cell surface (Eltzschig, 2013; Longhi et al., 2013). Under normal physiologic conditions, Adenosine generated in the extracellular space serves to protect the tissues against excessive inflammation and promotes repair mechanisms. Its level is relatively low and certainly below the sensitivity threshold of the various immune cells. However, during the hypoxic conditions, that normally characterize the tumor microenvironment (TME), Hypoxia Inducible Factor (HIF-1α) activates CD73 and CD39 and results in marked upregulation of extracellular Adenosine levels (Ohta, 2016). Hypoxia in the TME is, on one hand, the result of the increased oxygen demand of the proliferating tumor cells, and on the other hand, related to the inefficient blood supply offered by the tortuous tumor vasculature. In that harsh environment, Adenosine is usually present at high concentrations and is a crucial mediator of tumor cell growth as well as evasion of the antitumor immune response (Antonioli et al., 2013; Longhi et al., 2013; Ohta, 2016). Adenosine mediates its physiological functions through its interaction with four G-protein-coupled receptors A1, A2A, A2B, and A3 (Gessi et al., 2011). Although all adenosine receptors now have an increasing number of recognized biological roles in tumors, it seems that the A2AR is the most expressed on immune cells and both A2A and A3 subtypes are the most promising regarding drug development (Fredholm et al., 2001; Leone & Powell, 2015).

Activation of A2A receptors results in an immunosuppressive effect and evasion of anti-tumor immune response via several mechanisms. Their activation on human or mouse T cells was shown to decrease the secretion of various Th1/Th2 cytokines including, but not limited to, IL-2, IL-4, IL-5, IFN-γ and TNF-α (Allard et al., 2016). Moreover, A2AR activation resulted in a decreased T-cell proliferation as well as activation and induction of profound T cell apoptosis (Longhi et al., 2013). Furthermore, stimulation of A2AR on the surface of T-cells has also lead to downregulation of T-cell receptor expression (Hatfield & Sitkovsky, 2016). A2A receptors also have an inhibitory effect on the cytotoxic activity of NK cells. Indeed, the activation of these receptors resulted in the inhibition of perforin-mediated and CD95 ligand-mediated lysis of tumor cells. Their effect also further extends to a suppressive effect on anti-tumor M1 macrophages with decreased MHCII and IL-12 expression and increased IL-10 expression (Allard et al., 2016; Antonioli et al., 2013).

In addition to its effect on the anti-tumor immune response, Adenosine can also have a direct effect on tumor cell growth and proliferation. Stimulation of Adenosine receptors on the tumor cells inhibited tumor cell apoptosis, enhanced angiogenesis and promoted tumor metastatic potential (Antonioli et al., 2013; Ohta, 2016) . A number of studies have investigated the potential role of targeting Adenosine pathway in order to enhance the anti-tumor immune response. This was done either by targeting CD39 or CD73 to decrease Adenosine production in the tumor milieu or by Targeting A2A receptors to restore the anti-tumor function of the different immune cells in the TME (Beavis et al., 2015; Häusler et al., 2014).

A2A receptors antagonists have been developed and studied for the treatment of Parkinson's disease and they were found to be safe and well-tolerated in clinical trials. In recent years, they have also entered phase I

clinical trials for oncology (NCT02403193 and NCT02655822) (Beavis et al., 2015).

To the best of our knowledge, the expression profiles and clinical significance of A2A receptors in human breast cancer tissue has not been fully investigated. Moreover, little information is available about the prognostic effect of tumor A2AR expression and its association with other clinicopathological parameters. In the present study, we evaluated A2AR expression in surgically resected breast cancer tissue. Associations between their expression and the different clinicopathological parameters, as well as patients' prognoses, were analyzed.

MATERIALS AND METHODS

Patients and specimen collection

The present study was conducted on 30 formalin-fixed paraffin-embedded (FFPE) breast cancer tumor specimens from Egyptian patients who have been diagnosed using a US-guided biopsy followed by curative surgical treatment in Alexandria university hospitals, Egypt. Written informed consent to use these specimens for medical research was obtained from all patients. Clinical and pathological data were retrospectively retrieved by the review of the patients' records.

Two independent pathologists reviewed the tumor specimens to confirm the tumor type, Nottingham grade, TNM stage, mitotic count, lymphovascular invasion, and presence or absence of in situ tumor component. The percentage of tumor-infiltrating lymphocytes (TILs) was also assessed in the H&E stained sections by 2 independent pathologists blinded from the clinical information and an average percentage was agreed upon based on the criteria proposed by the International TILs Working group (Salgado et al., 2014). Data concerning both the hormone receptor status (ER, PR) and the HER2 expression for each tumor were retrieved from the patients' records. Patients' followed up data (tumor recurrence, metastasis as well as patient survival) after a period ranging between 2 and 5 years were also recorded.

Immunohistochemistry procedures and interpretation

Unstained slides of FFPE tumor tissue were used for the immunohistochemical staining. Representative samples were stained using A2AR primary mouse monoclonal antibody (dilution 1:100, clone SC-32261, Santa Cruz Biotechnology, USA), Ki67 monoclonal antibody (dilution 1:200, MIB1 clone, Dako, Denmark), as well as basal-like markers CK5 Rabbit monoclonal antibody (dilution 1:100, Abcam, ab52635) and CK6 mouse monoclonal antibody (dilution 1:100, Abcam, ab18586). Positive and negative controls were included in all the runs. All slides were deparaffinized in xylene then rehydrated in descending concentrations of alcohol. Heat-mediated antigen retrieval in citrate buffer was performed using a microwave before starting the immunohistochemical staining protocol. The streptavidin-biotin–peroxidase complex method was used. This technique involves sequential incubation of the specimens with an unconjugated primary antibody specific to the target antigen, a biotylinated secondary antibody that reacts with the primary antibody, enzyme-labeled streptavidin, and DAB substrate chromogen. The Abcam Detection kits (Mouse specific HRP/DAB (ABC) detection IHC kit, ab64259) and (Rabbit specific HRP/DAB detection kit, ab64261) were used.

For A2AR staining, semi-quantitative (Modified H-score) method was used to assess the degree of positive staining. This method assigns an IHC H-score to each patient on a continuous scale of 0–300, based on the percentage of positively stained cells at different staining intensities (Pirker et al., 2012). In this method, membranous and cytoplasmic staining of A2ARs was scored according to four categories: 0 for 'no staining', 1 + for 'light staining visible only at high magnification', 2 + for 'intermediate staining' and 3 + for 'dark staining, visible even at low magnification'. The percentage of cells at different staining intensities was determined by visual assessment, with the score calculated using the formula 1 × (% of 1 + cells) + 2 × (% of 2 + cells) + 3 ×(% of 3+cells) depending on the percentage of stained cells. Stromal lymphocytes that exhibited positive staining were used as an internal positive control.

Ki-67 scoring was performed by both semi-quantitative assessments (visual counting and image analysis). As for the semi-quantitative assessment of Ki-67; three fields each containing 100 malignant cells were evaluated at a time and an average was calculated. The number of brown-stained nuclei regardless of intensity is designated as a fraction out of the total number of malignant nuclei (Inwald et al., 2013). A Ki-67 cut-off point of 20% was defined according to previous recommendations (Bustreo et al., 2016). Image analysis for Ki-67 was carried out using ImmunoRatio® plugin which is part of ImageJ software. Two images per tumor were captured using Olympus microscope equipped with a color camera and submitted for analysis.

CK5/6 IHC was performed using CK5 rabbit monoclonal antibody (dilution 1:100, Abcam, ab52635) and CK6 mouse monoclonal antibody (dilution 1:100, Abcam, ab18586). Basal cell carcinoma was used as a positive control. Intermediate to strong cytoplasmic staining in more than 10% of the cells were considered positive, weak to intermediate staining in less than 10% of the cells was considered focally positive and no staining was considered negative.

Statistical analysis

Data were analyzed using the Statistical Package for Social Science (SPSS) program (version 21). Data were entered as numerical or categorical as appropriate. Data were described using minimum, maximum, mean, standard deviation and 95% confidence interval (95%CI) of the mean for the normally distributed. Categorical variables were described using frequency and percentage of the total. For non-normally-distributed data, comparisons between two studied independent groups were carried out using the Mann-Whitney U test.

Comparisons were carried out between more than two independently studied not normally-distributed subgroups using Kruskal-Wallis test. Chi-square test was used to test the association between qualitative variables. Fischer's exact test and Monte Carlo correlation was carried out when indicated. An alpha level was set to 5% with a significance level of 95%, and a beta error accepted up to 20% with a power of study of 80%. Recurrence data were analyzed using the Kaplan–Meier estimator method. The log-rank test was used for the comparison of recurrence curves. Statistical significance was set at $p < 0.05$.

RESULTS

The present study comprised 30 BC patients. Eighteen (60%) of whom were less than 50 years of age and 12 (40%) were 50 years of age and above. Histopathologic evaluation of the tumors revealed that 28/30 (93.3%) cases were invasive ductal carcinomas, no special type (IDC, NST) and two were classified as invasive lobular carcinoma (ILC). As for the hormone receptor expression, 19 cases (63.3%) were ER-positive whereas 11 (36.7%) were ER-negative. Twenty-one patients (70%) were HER2 negative and 9 (30%) were HER2 positive by immunohistochemistry and FISH analysis.

Based on the review of the tumors' hormonal profiles from the patients' records and based on the interpretation of the immunohistochemical staining of CK5, CK6, and Ki-67 proliferation index, Tumors were molecularly subclassified into Luminal A (10 cases), luminal B (13 cases), Her-2 enriched (1 case), Triple-negative (6 cases), and basal-like (0 case). The percentage of tumor-infiltrating lymphocytes in each tumor was evaluated and tumors were classified as mild (up to 5%), moderate (5-9%) and strong (more than 10%) (Lee et al., 2015) (Figure 1).

Adenosine receptors (A2AR) were expressed not only on the tumor-associated immune cells (Figure 3) but also on the tumor cells themselves. A2AR were expressed in all examined tumor specimens but with varying staining intensities. Eight (8) out of 30 cases (26.7%) showed weak A2A expression (H-score below 100) whereas the remaining 22 cases (73.3%) showed moderate to strong expression A2AR expression (H-score between 100-300) (Figure 2 a, b, c). A2AR expression on the tumor cells was significantly higher in younger patients compared to older patients where 88.8% of the young patients (< 50ys) showed strong expression of A2AR compared to only 50% of the older patients showing strong expression of A2AR ($p = 0.018$, Chi-square test).

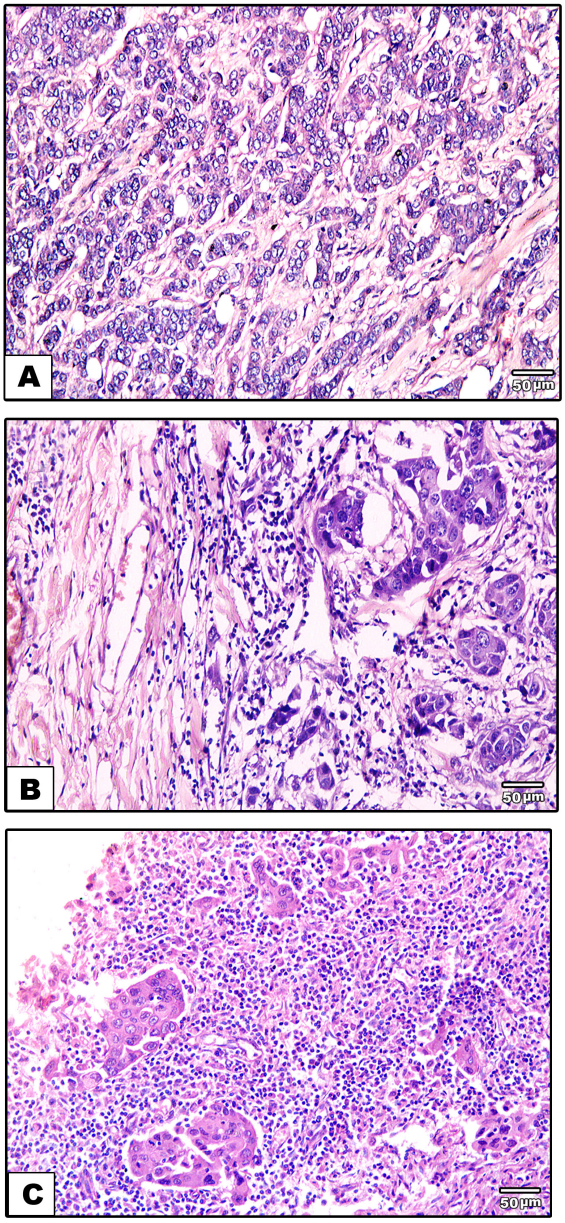

Figure 1. Microscopic picture of breast cancer tumors showing variable infiltration with tumor-infiltrating-lymphocytes (TILs) in the studied tumor specimens. (A) No lymphocytes within the tumor tissue. (B) Moderate infiltration with TILs (9%). (C) Marked infiltration with lymphocytes (80%).

Tumors molecularly classified as “Luminal B”, “triple-negative breast cancer (TNBC)” and “HER2 enriched” subtypes showed a higher A2AR expression compared to luminal A tumors (p=0.028, Pearson chi-square test). A significant association was also noted between A2AR expression and a high Mitotic score (p = 0.013, Pearson chi-square test) as well as a high proliferative index (as evidenced by a high Ki-67 expression > 20%) (P = 0.018, Pearson’s chi-square test).

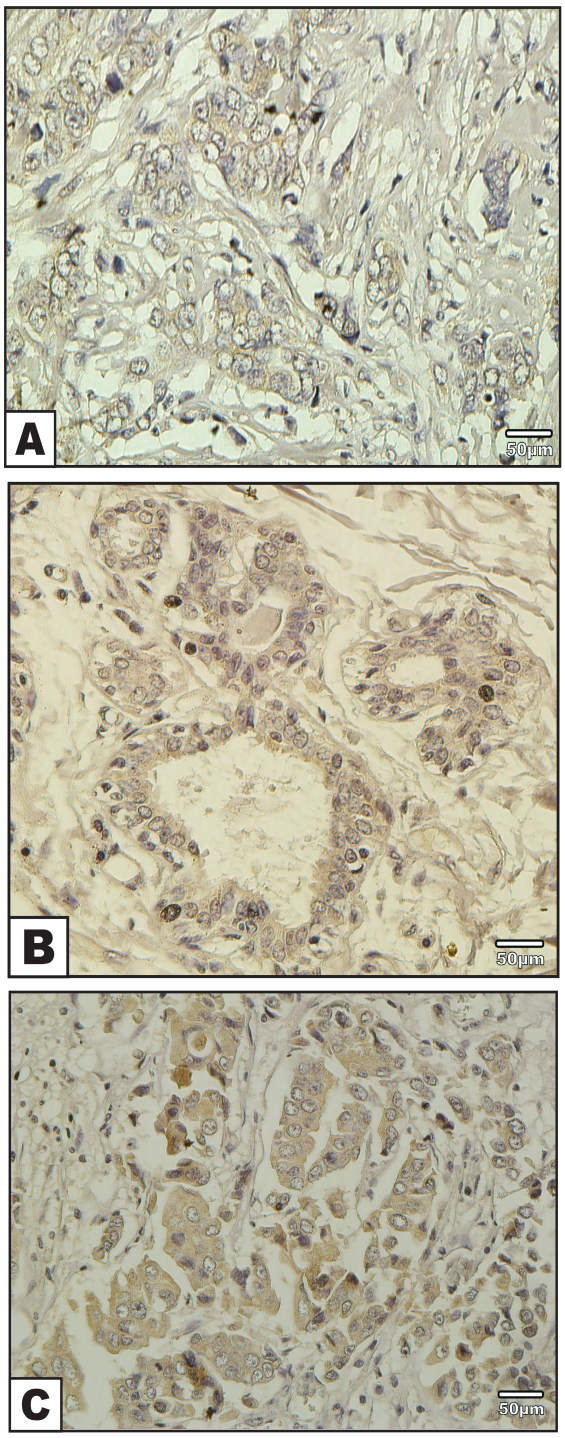

Figure 2. Microscopic evaluation of Immunohistochemical expression of A2ARs in the studied specimens. (A) Mild membranous expression of A2A receptors (H-score 60) on the surface of the tumor cells. Note that the intensity of staining is very mild and only noted on high magnification. (B) Moderate expression of A2A receptors. A well-differentiated tumor formed of variable-sized tubules shows a moderate membranous expression of A2A receptors (H-score 180). (C) A moderately differentiated tumor formed of nests of malignant cells all showing strong expression of A2AR in all tumor cells. (H-score, in this case, was 300).

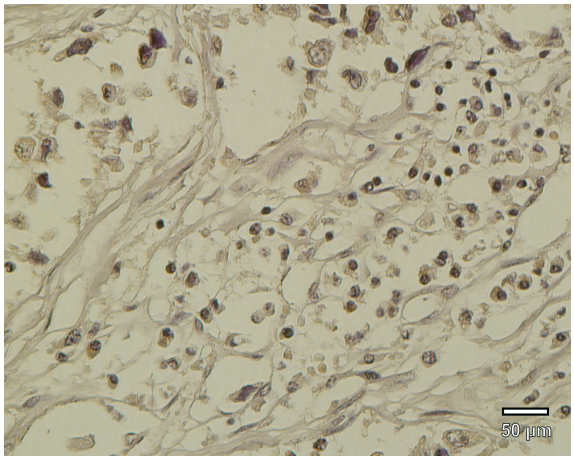

Figure 3. Microscopic picture showing A2A receptor expression on the tumor-infiltrating lymphocytes (TILs) in breast cancer.

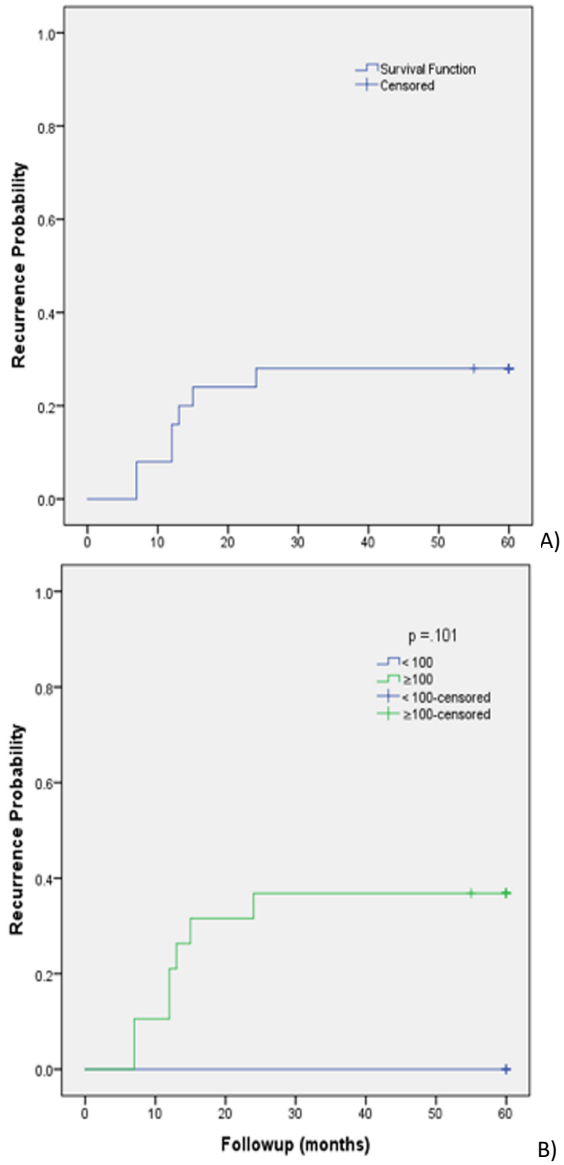

Figure 4. Kaplan–Meier estimates for the entire cohort of (A) Overall recurrence, and (B) Recurrence stratified by A2A H-score.

A2ARs were also strongly expressed in most tumors showing Progesterone receptor expression and those with HER2 overexpression. (p= 0.031 Chi-square test).

No statistically significant association was found between A2AR expression and Tumor size, lymph node status, tumor type, tumor grade, lymphovascular invasion, the presence of an in-situ component or the percentage of tumor-infiltrating lymphocytes (TILs). (Table 1).

Kaplan–Meier estimates for overall recurrence was 27% at 5 years. Overall recurrence for patients who had a strong expression (≥100) was approximately 37% at 5 years. Those who had weak expression (<100) overall recurrence was about 0%, yet there was no statistically significant difference noted, p= 0.101 (figure 4 a, b). Therefore, the expression of A2AR did not show any statistically significant association with recurrence, metastasis or patient survival when these patients were followed up for a period between 2 and 5 years after treatment.

DISCUSSION

The treatment of breast cancer has achieved great success in the past decade leading to a dramatic improvement in patient overall survival (Miller et al., 2016; Siegel et al., 2016). Nevertheless, there is still a continuous search for novel therapeutic targets that would offer these patients a better outcome and an improved quality of life.

In recent years, the use of immune checkpoint inhibitors has emerged as a highly promising treatment modality in various types of cancers. PD-L1 monoclonal antibodies are currently used in addition to chemotherapy for the treatment of advanced malignant melanoma (Johnson et al., 2015).

However, the role of these immunotherapy molecules in the treatment of breast cancer is still being investigated. In our study, we investigated the expression of another immune checkpoint molecule, Adenosine receptors (A2AR) in different molecular subtypes of breast cancer. To the best of our knowledge, this is the first study to investigate the clinicopathological implications of A2ARs expression in breast cancer.

Ohta and Sitkovsky showed for the first time in 2001 that A2A receptor-deficient mice were unable to control inflammation resulting in an exaggerated immune response and extensive tissue disruption and cell death (Ohta & Sitkovsky, 2001). Since that time, the Adenosine receptor pathway has emerged as a crucial "immune checkpoint" and in-vitro studies have successively proved that stimulation of A2A receptors on immune cells like T-cells, NK-cells and dendritic cells inhibited the activity of these cells thus creating an immunosuppressed niche that is capable of evading the body's immune response (Morello et al., 2009).

In our study, we confirmed for the first time that A2A receptors were expressed not only on tumor immune cells but also highly expressed in the breast cancer tumor cells themselves. We demonstrated that their expression was significantly higher in young aged patients (< 50 years) compared to older ones (older than 50years of age) and also was associated with a high mitotic score and a higher proliferative index in the studied cases. This finding suggests a possible role for these receptors in enhancing tumor cell proliferation and promoting more aggressive behavior.

The present study has also demonstrated that A2ARs are more likely to be show strong expression in the more aggressive molecular subtypes of breast cancer (Luminal B, HER-2 enriched and TNBC) compared to the more indolent luminal A breast cancers. These tumors subtypes are also known to show less response to current treatment modalities. It has been previously suggested that younger women were more likely to present with the more aggressive tumor subtypes like TNBC compared to older females, aged 60 years and above, who most likely present with luminal A subtype rather than with TNBC which is known to be the least common in this age group (McGuire et al., 2015).

Our findings, as well as those previous ones, has lead us to speculate that the expression of A2AR on tumor cells could be an important factor leading to enhanced tumor cell proliferation and thereby a more aggressive tumor phenotype.

It is well documented that A2AR stimulation on immune cells has an immunosuppressive effect (Allard et al., 2016; Antonioli et al., 2013; Longhi et al., 2013). However, very few studies have investigated the effect of A2AR stimulation on the tumor cells themselves.

One *in-vitro* study has suggested that adenosine receptor expression on the tumor cells may directly enhance tumor cell growth (Bavaresco et al., 2008). Another similar study investigated the effect of adenosine on *in-vitro* cultured tumor cells and showed that the addition of adenosine enhanced cell growth whereas co-treatment with A2AR antagonists had an opposite effect thus proving that this growth-promoting effect is mediated via A2A receptors (Bavaresco et al., 2008). Another study has also shown that A2AR stimulation by adenosine antagonists was associated with increased tumor cell apoptosis (Mediavilla-Varela et al., 2013). Furthermore, it was found that inhibition of the enzymatic activity of CD73, on the tumor cells, resulted in a reduction of adenosine level with inhibition of tumor cell proliferation and enhanced apoptosis (Zhi et al., 2010).

A2ARs were also found to be expressed in the tumor cells in different other tumors; such as neuroblastoma, monocytic lymphoma, T-cell leukemia, melanoma, epidermoid cells, colon carcinoma, and human breast cancer MCF-7 cells (Gessi et al., 2011). However, the exact effect of A2A receptor stimulation was found to be different from one tumor type to the other. For instance, in human A375 melanoma cells and Caco-2 human colonic cancer cells, A2Areceptors activation was associated with a caspase-9 and caspase-3 mediated apoptotic cell death following mitochondrial damage (Merighi et al., 2002; Yasuda et al., 2009). Whereas, stimulating A2A receptors by agonists stimulated the proliferation of MCF-7 cells (Etique et al., 2009). Nevertheless, there is still not enough research studying the in-vivo effect of A2A receptors in breast cancer within the tissue microenvironment and the exact mechanisms downstream these receptors in breast cancer cells has not yet been fully established (Gessi et al., 2011). An *in vivo* study on CD73-knockout mice studying B16F10 melanoma has suggested a possible role for

Table 1. Association of A2AR expression with clinicopathological features of the tumors

	No (%)			*p*-value
	A2A Receptor Expression (H-score <100) (8)	**A2A Receptor Expression (H-score ≥100) (22)**	**Total (%)**	
Age group (years) n=30				*p* = 0.018*
≤50y	2 (11.11%)	16 (88.88%)	18 (60%)	
>50y	6 (50%)	6 (50%)	12 (40%)	
Tumor size n=30				*p* = 0.361
T1/T2	4 (21.05%)	15 (78.94%)	19 (63.33%)	
T3/T4	4 (36.36%)	7 (63.63%)	11 (36.66%)	
Lymph node status n=29				*p* = 0.495
Negative (N0)	2 (40%)	3 (60%)	5 (16.66%)	
Positive (N1/ N2/N3)	6 (25%)	18 (75%)	24 (83.33%)	
Tumor Grade n=30				*p* = 0.783
G1	1 (33.33%)	2 (66.66%)	3 (10%)	
G2/3	7 (25.92%)	20 (74.07%)	27 (90%)	
Mitotic score n=30				*p* = 0.013*
1	6 (46.15%)	7 (53.84)	13 (43.33%)	
2	1 (6.25%)	15 (93.75%)	16 (53.33%)	
3	1 (100%)	0 (0.00%)	1 (3.33%)	
ER expression n=30				*p* = 0.098
Positive	7 (36.84%)	12 (63.15%)	19 (63.3%)	
Negative	1 (9.09%)	10 (90.90%)	11(36.6%)	
PR expression n=30				*p* = 0.031*
Positive	8 (38.09%)	13 (61.90%)	21(70%)	
Negative	0 (0.00%)	9 (100%)	9 (30%)	
Her-2 expression n=30				*P* = 0.031*
Positive	0 (0.00%)	9 (100%)	9 (30%)	
Negative	8 (38.09%)	13 (61.90%)	21(70%)	
Ki-67% n=30		31		*p* = 0.018*
≤20%	6 (50%)	6 (50%)	12 (40%)	
>20%	2 (11.11%)	16 (88.88%)	18 (60%)	
Histological type n=30				*p* = 0.377
IDC, NST	8 (28.75%)	20 (71.42%)	28 (93.33%)	
ILC	0 (0.00%)	2 (100 %)	2 (6.66%)	
Molecular subtype n=30				*p* =0.028*
Luminal A	6 (60%)	4 (40%)	10 (33.33%)	
Luminal B	2 (15.38%)	11 (84.61%)	13 (43.33%)	
Her-2 enriched	0 (0.00%)	1 (100%)	1 (3.33%)	
TNBC	0 (0.00%)	6 (100%)	6 (20%)	
Basal-like	0 (0.00%)	0 (0.00%)	0 (0.00%)	
Intra-tumoral Lymphocytes (ITLs) n=30				*p* = 0.536
0/1	7 (29.16%)	17(70.83%)	24 (80%)	
2/3	1 (16.66%)	5 (83.33%)	6 (20%)	
Outcome n=30				*p* = 0.222
No Recurrence/metastasis	6 (35.29%)	11 (64.70%)	17 (56.66%)	
Recurrence /metastasis	2 (15.38%)	11 (84.61%)	13 (43.3%)	

adenosine through its various receptors in controlling tumor vascularization with a possible mitogenic effect on endothelial cells (Koszałka et al., 2016). Thus, adding to the multifaceted role of adenosine and its receptors in controlling tumor growth.

Among our studied cases, only two of the patients who showed low A2AR expression developed recurrences/metastasis during the follow-up period, yet this did not prove to be statistically significant nor did the expression of A2ARs show any significant association with overall survival in our patients.

In contrast to our findings, a study on non-small cell lung cancer (NSCLC) showed that A2ARs was highly expressed in 49.2% of the cases and their expression was associated with a significantly better overall survival that is recurrence-free in patients with high A2AR expression compared to those with low A2AR expression (Inoue et al., 2017). This, in turn, supports the notion that the role of these receptors may differ from one tumor to another. Therefore, more studies are still needed to further elucidate their effect *in-vivo*. Whereas the use of A2AR antagonists in Lung adenocarcinoma induced apoptotic cell death (Mediavilla-Varela et al., 2013), their effect on breast cancer cells has not been fully investigated. Therefore, it is highly recommended that A2AR antagonists be further tested on different breast cancer cell lines *in-vitro* as well as *in -vivo* models to further demonstrate their effect on tumor cell survival, proliferation, and migration.

Moreover, studies are also still needed to determine the relevant downstream signaling pathways through which A2A receptors exert their effect on tumor cells not only immune cells. Understanding the signaling pathways involved could guide more rational combinations of chemotherapeutic agents with A2AR antagonism to improve the therapeutic outcome.

CONFLICT OF INTEREST

All authors declared no conflicts of interest.

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