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Investigation of CD73 expression in Iraqi patient women with breast tumors

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ABSTRACT

Biomarkers that aid in the diagnosis, prognosis, and prediction of breast cancer are critical for early detection and effective disease management throughout treatment. Although preclinical studies suggest that 5-ectonucleotidase (CD73) could be a diagnostic marker in several types of cancer, it can be an appealing treatment strategy in some molecular subgroups of breast cancer. The aim of this study is to investigate the expression of CD73 in breast tumors (malignant and benign) in Iraqi patients using immunohistochemistry (IHC), as well as its correlation with clinicopathological characteristics. A total of eighty-nine samples of paraffin embedded tissue blocks of different breast tissue tumors (71 females and 18 were males) with their data in addition to eleven cases of healthy breast biopsies as a control were collected from public and private hospitals and health institutions in Baghdad. The findings of this task demonstrated substantial variations in the expression of CD73 between breast malignant, benign tumors and control groups ($P < 0.001$). CD73 expression was highest in 75.56% of malignant cases, while only 31.82% of benign cases showed positive expression of CD73. Furthermore, significant associations were found between CD73 with gender and histological types in benign cases and with (ER, PR, HER-2) negative and triple negative molecular subtypes in malignant cases. There were no significant variations in CD73 expression with other clinicopathological features. This allowed us to suggest that inhibiting CD73 could be an appealing treatment strategy in the TNBC subgroup.

Keywords: *Breast Tumors, Ecto-5'-nucleotidase, CD73, Immunohistochemistry (IHC).*

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INTRODUCTION

Breast cancer (BC) is the most common cancer in women and the second most common cancer among newly diagnosed cancers globally[1]. It is the first of the top 10 malignant neoplasms harming the community in Iraq[2]. Breast tumors can develop in a variety of locations in the breast, including the ducts, lobules, or tissue in between. Across the different ranges of breast carcinoma, there are variously identified types of BC based on their invasiveness in comparison to the primary tumor[3]. BC can be classified as invasive or non-invasive based on its relationship to the basement membrane[4]. Early stage breast cancer has no symptoms and is only identified at advanced stages, when treatment is rendered ineffective. It has a bad prognosis and increases the risk of high mortality in women[5]. The capability of cancer cells to spread from a primary lesion to distal organs is the leading cause of death in cancer patients. A variety of cellular mechanisms are involved in the dissemination of cells from primary tumors. This include invading through or partnering with the stroma is one example, as is avoiding immune surveillance by suppressing or co-opting their anti-tumorigenic pathways, evading and modifying the tissue microenvironment, and evolving resistance to therapeutic intervention[6]. Many factors, either individually or collectively, contribute to the onset of BC, Particularly in women with a genetic predisposition to the disease or who are exposed to high-risk factors[7]. In addition, the tumor phenotype in terms of molecular subtype in BC patients differs based on gene expression profiles: luminal epithelial/estrogen receptor (ER) positive, Human epidermal growth factor receptor (HER2) positive, triple negative, and normal breast-like[8]. Early diagnosis of BC emphasizes the search for, development of, and optimization of diagnostic biomarkers that can improve prognosis and therapy results. Cancer diagnostics is undergoing a paradigm shift with the addition of molecular biomarkers to conventional diagnostic assays. The molecular changes include those involving DNA, RNA, microRNAs (miRNAs), and proteins[9]. A useful biomarker should not only predict prognosis but also responsiveness to therapy[10]. Treatment consists of a BC multimodal approach that includes neoadjuvant chemotherapy, surgery for operable tumors, radiation, adjuvant chemotherapy, and/or endocrine therapy[11].In recent years, CD73 has been widely studied as a cancer therapy target[12]. And in vitro, anti-CD73 antibody treatment has consistently demonstrated an efficient reduction of BC cell growth and invasion[13]. CD73, also known as ecto-5'-nucleotidase, is a glycosylated protein that is connected to the plasma membrane's external surface by a glycosylphosphatidyl inositol anchor and is overexpressed in a number of malignancies, including BC, ovarian cancer, and renal cell cancer[14].Aside from its enzymatic function, CD73 is a regulatory molecule linked to cancer spreading and aggressive features. This enzyme is important in extracellular purinergic signaling because it generates adenosine from AMP[15].

MATERIALS & METHODS

Materials

A total of eighty-nine of paraffin embedded tissue blocks of different breast tissue tumors (71 females and 18 were males) with their data were collected from archive of Histopathology Department/ Teaching Laboratories of Medical City, Al-Yarmouk Teaching Hospital, and a private Laboratory in Baghdad-Iraq, for the years (2018-2021). The patients' cases were distributed as follows: Forty-one cases were females' malignant breast lesions, thirty cases were females' benign, fourteen cases were males' benign and the last four cases were males' malignant tissues. Clinical information including age, gender, tumor site, tumor size, lymph node metastasis, histopathological types, grade, stage and (ER, progesterone receptor (PR) and HER2) were obtained from reviewed patients' medical records and pathologic report. In addition, eleven cases of health breast biopsies were collected from Forensic Medicine Department in Baghdad as a control.

Methods

Immunohistochemistry staining protocol

The immunohistochemistry (IHC) was carried out using the generic protocol according to (Magaki et al., 2019)[16] and abcam protocol instructions. The tissue sections were de-waxed by incubation of slides in oven (Memmert SN10), heated in 75-80 °C for 25 minutes, then quick dips in xylene and returned to oven for 5min. Next, sections were rehydrated by dipping in decreasing concentrations of alcohol.

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The slides were then dipped in the antigen retrieval solution and placed in a water bath that had been heated to 95 °C and allowed to boil for 45 minutes. After that, the slides were treated with a hydrogen peroxide block solution for 15 minutes. Primary antibody of CD73 (ab133582) were diluted to 1:100 and applied to the slides and incubated for 2hrs. in room temperature in a humidified chamber. After washing the slides, the secondary antibodies Goat Anti-Rabbit HRP Conjugate (abcam236469) were added and applied to the slides by adding 2-3 drops and incubated in a humidified chamber for 30 minutes in room temperature. Then, DAB Chromogen and its substrate applied to slides in a humidified chamber and incubated for 7-10 minutes in room temperature until the brown staining development was visualized. Then, slides were washed with TBS 4 times for 1minutes per each. Slides then were counterstained by adding drops from Mayer' Hematoxylin for 1-2minutes, then carefully washed in tap water until ran clear. Next, slides were dehydrated by immersing in increasing concentrations of alcohol and cleared by immersing in xylene (2*2minutes). Finally, slides were mounted and cover slipped using DPX mounting solution.

Scoring system of IHC

The scoring system for CD73 expression was done according to (Choi et al., 2022)[13] and the results were assessed and read by a specialist pathologist. Each tumor was given a grade on a scale of 0–3 based on membrane staining (with or without cytoplasmic staining) intensity and ratio of positive tumor cells. The range of positivity was scored as (0, no staining); (1+, <30% of cancer cells staining); (2+, 31–60%) and (3+, >60%). IHC staining for CD73 in BC tissue was classified as negative expression for (score 0 and 1) and positive expression for (score 2 and 3).

Statistical analyses

The Statistical Analysis System SPSS-22 was used to detect the effects of different factors in study parameters. Data was presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The Chi-square test was used to significant comparison between percentages in the different groups. Probability in this study: Significant (P≤0.05) *, highly significant (P≤0.01) ** and NS (non-significant).

RESULTS

The positive expression of CD73 was shown as brown membrane staining (with or without cytoplasmic staining). The findings revealed that CD73 was expressed positively in 75.56% of malignant cases and 31.82% of benign specimens, while it was expressed negatively or not at all in 100% of normal breast duct tissues Table 1, Fig. 1, and Fig. 2.

TABLE 1. Positivity of CD73 for breast tissue samples.

CD 73 Expression Score		Malignant		Benign		control		P value
		N	%	N	%	N	%	
Positive		34	75.56	14	31.82	0	0.00	< 0.001**
Negative		11	24.44	30	68.18	11	100.00	
	Significant differences between proportions using Pearson Chi-square test at 0.05 level. * (significant), ** (high significant) and NS: Non-Significantly.							

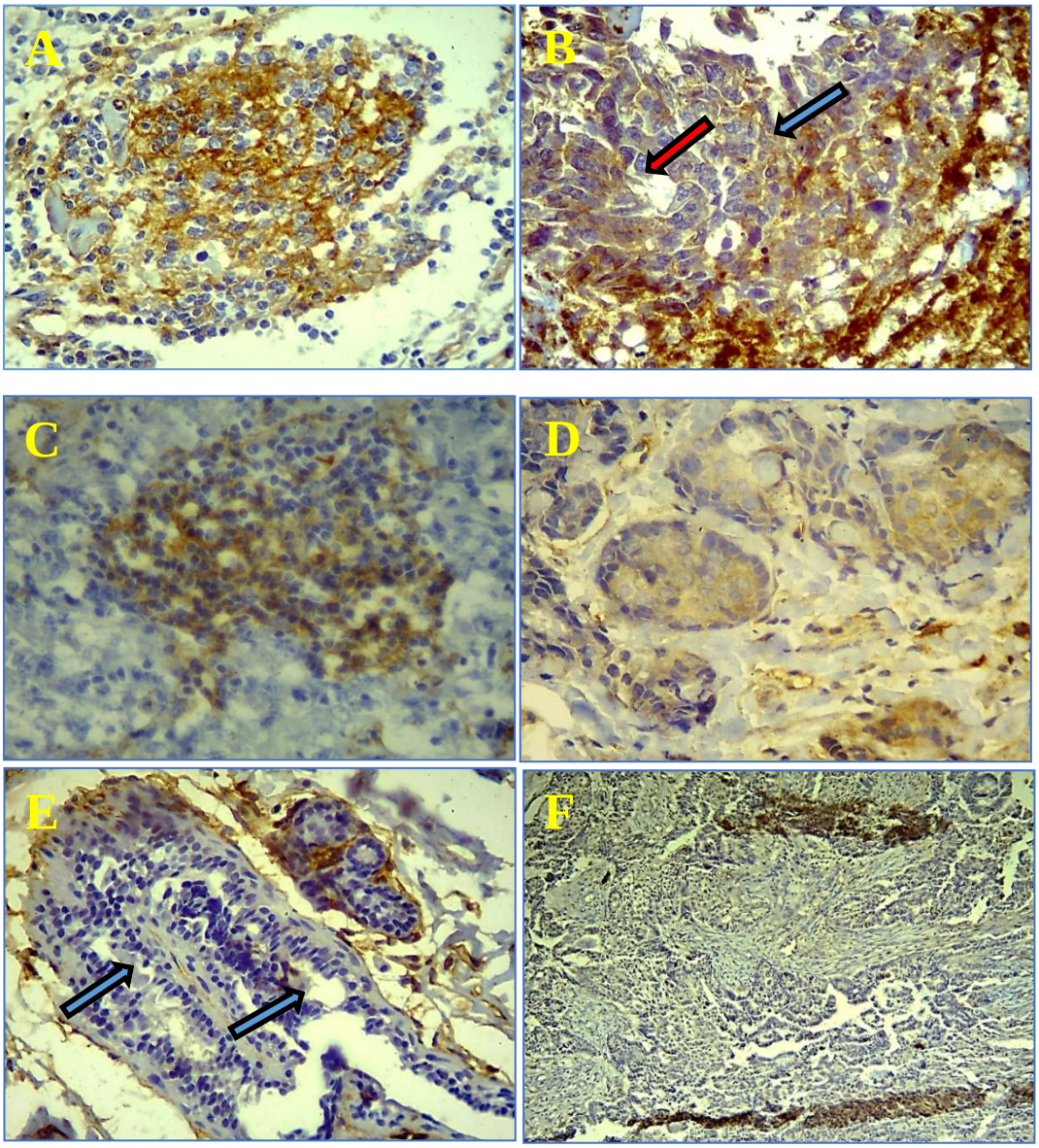

FIG. 1. Immunohistochemical assessment of CD73 for malignant cases

A (female case): strong membranous and cytoplasmic expression of CD73 in moderately differentiated IDC, grade II/stage IIB, score +3, (brown area) (40X). B (female case): Strong membranous and cytoplasmic reactivity of CD73 in high grade ductal carcinoma, grade III/stage IIIA, score +3, (brown color - membranous reactivity (blue arrows) and membranous with cytoplasmic reactivity (red arrows)), (40X). C (female case): strong membranous and cytoplasmic expression of CD73 in invasive breast carcinoma, and score +3, (brown area) (40X). D (female case): Weak membranous and cytoplasmic reactivity of CD73 in IDC & DCIS, grade II, and score +2, (brown area), (40X). E (female case): Strong membranous and cytoplasmic reactivity of CD73 in IDC of a female case, grade II, and score +1, (brown area-blue arrays), (40X). F (male case): Strong staining, membranous and cytoplasmic expression of CD73 in IPC, grade II, and score +2, (brown area) (10X).

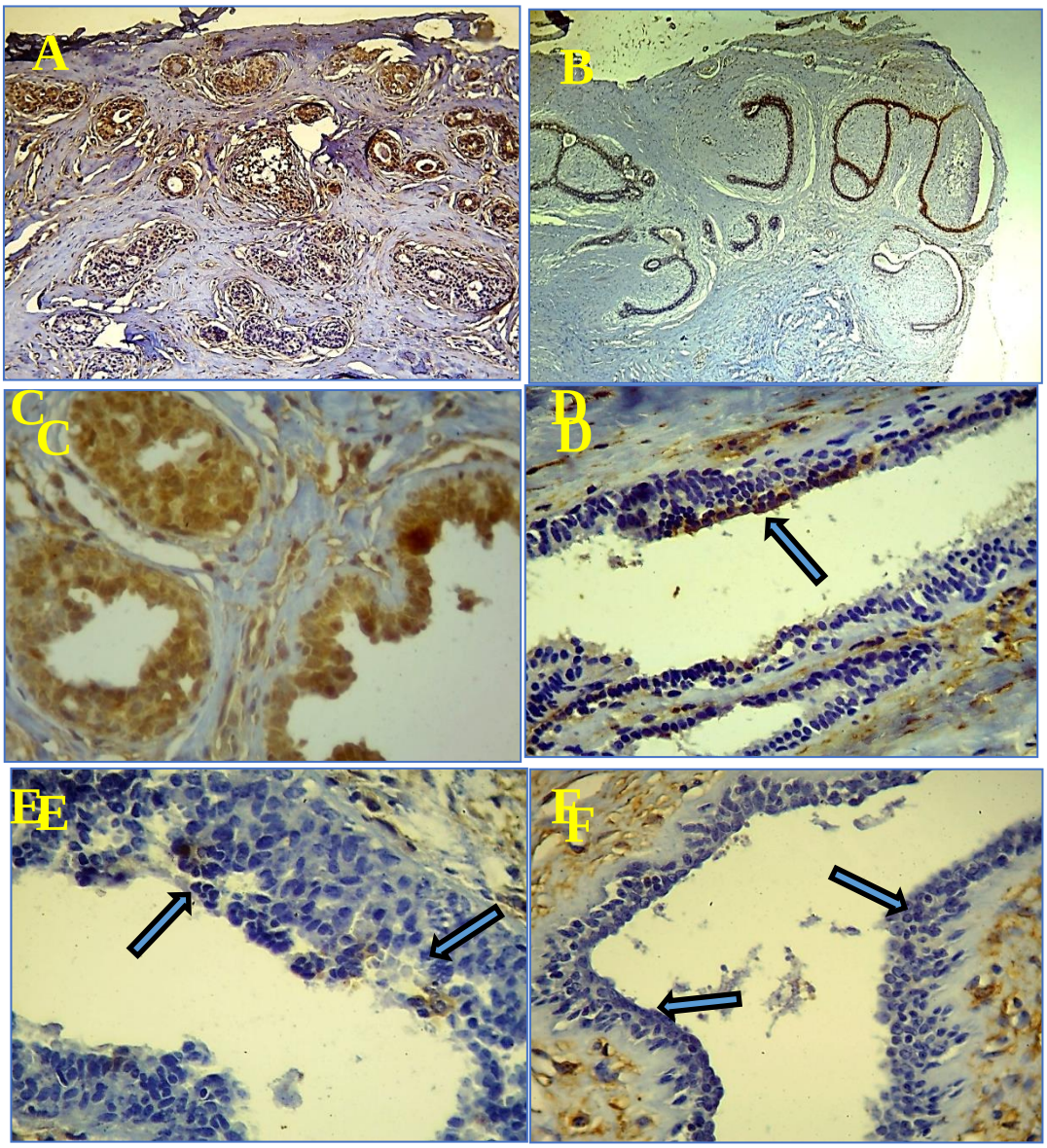

FIG. 2. Immunohistochemical assessment of CD73 for benign cases .

A (female case) : Strong staining , membranous and cytoplasmic expression of CD73 in Fibrocystic changes with ductal hyperplasia without atipea , score +3 , (brown areas) (10X). B (female case): Strong membranous and cytoplasmic reactivity of CD73 in fibroadenoma , score + 3 (brown area) , (4X). C (male case): Strong staining , membranous and cytoplasmic expression of CD73 in fibrocystic changes , score +3 , (brown areas) (40X). D (male case): Strong staining , membranous and cytoplasmic expression of CD73 in ductal epithelial hyperplasia with prominent stromal seclerosis , score +2 , (brown area - blue arrays) (40X). E (male case): Weak staining , membranous and cytoplasmic expression of CD73 in Florid ductal hyperplasia with focal atypia , score + 1 (Negative expression) , (brown area - blue arrays (40X). F : Negative control showing female normal breast duct cells with no expression of CD73 (blue stain cytoplasmic and cells membranes - blue arrows) (40X) .

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Association of IHC expression of CD73 with clinicopathological features

1- Association of IHC expression of CD73 with age

The present study found no significant correlation between CD73 expression with patients' ages in all studied groups (P = 0.793 for malignant cases, P = 0.517 for benign cases) Table 2.

TABLE 2. Association of IHC expression of CD73 with age.

Score of CD73	Malignant								Benign							
	Age								Age							
	<40		40-49		50-59		60 ≥		<40		40-49		50-59		60 ≥	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Score 0	0	0.0	0	0.0	2	11.8	1	7.7	9	28.1	3	42.9	1	50.0	1	33.3
Score +1	1	25.0	2	18.2	3	17.6	2	15.4	14	43.8	2	28.6	0	0.0	0	0.0
Score +2	1	25.0	7	63.6	5	29.4	6	46.2	5	15.6	1	14.3	1	50.0	2	66.7
Score +3	2	50.0	2	18.2	7	41.2	4	30.8	4	12.5	1	14.3	0	0.0	0	0.0
P value	0.793 NS								0.517 NS							
Statistically significant differences between percentages using the Pearson Chi-square test at the 0.05 level. * (significant), ** (high significant) and NS: Non-Significantly.																

2- Association of IHC expression of CD73 with gender

The present study revealed that the expression of CD73 did not significantly correlate with gender in the studied group (P = 0.3 for malignant cases), but there were extremely high significant differences in benign cases (P = 0.001) Table 3.

TABLE 3. Association of IHC expression of CD73 with gender.

Score of CD73	Malignant				Benign			
	Female		Male		Female		Male	
	N	%	N	%	N	%	N	%
Score 0	3	7.14	0	0.00	13	43.33	1	7.14
Score +1	8	19.05	0	0.00	5	16.67	11	78.57
Score +2	18	42.86	1	25.00	7	23.33	2	14.29
Score +3	12	30.95	3	75.00	5	16.67	0	0.00
P value	0.3 NS				0.001**			
Statistically significant differences between percentages using the Pearson Chi-square test at the 0.05 level. * (significant), ** (high significant) and NS: Non-Significantly.								

3- Association of IHC expression of CD73 with site

The present study revealed no significant differences in the expression of CD73 (P= 0.827 for malignant, P= 0.289 for benign cases) between left and right breasts in groups of study Table 4.

TABLE 4. Association of IHC expression of CD73 with site.

Score of CD73	Malignant				Benign			
	Left		Right		Left		Right	
	N	%	N	%	N	%	N	%
Score 0	2	10.53	1	3.85	8	33.33	6	30.00
Score +1	3	15.79	5	19.23	6	25.00	10	50.00
Score +2	8	42.11	11	42.31	6	25.00	3	15.00
Score +3	6	31.58	9	34.62	4	16.67	1	5.00
P value	0.827 NS				0.289 NS			
Statistically significant differences between percentages using the Pearson Chi-square test at the 0.05 level. * (significant), ** (high significant) and NS: Non-Significantly.								

4- Association of IHC expression of CD73 with size

The present study found no significant correlation between CD73 expression and tumor diameter (P = 0.479 for malignant specimens, P = 0.467 for benign specimens) Table 5.

TABLE 5. Association between IHC expression of CD73 and tumor size

Score of CD73	Malignant						Benign					
	2 <		2—5		5 >		2 <		2—5		5 >	
	N	%	N	%	N	%	N	%	N	%	N	%
Score 0	0	0.00	3	13.64	0	0.00	6	37.50	6	25.00	2	50.00
Score +1	1	20.00	3	13.64	4	33.33	6	37.50	9	37.50	1	25.00
Score +2	3	60.00	9	40.91	3	25.00	4	25.00	4	16.67	1	25.00
Score +3	1	20.00	7	31.82	5	41.67	0	0.00	5	20.83	0	0.00
P value	0.479 NS						0.467 NS					
Statistically significant differences between percentages using the Pearson Chi-square test at the 0.05 level * (significant), ** (high significant) and NS: Non-Significantly.												

*It is worth noting that there are 3 malignant cases in which we do not know the size of the tumor because the samples were tru-cut biopsy.

5- Association of IHC expression of CD73 with histological types

The present study shows no significant differences (P = 0.422) between CD73 expression and tumor histology Table 6. While in benign cases, the revealed significant differences (P = 0.015) between the expression of CD73 and the histological type of malignancy. The benign (score +2) had the highest CD73-positive expression (33.33%) Table 7.

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TABLE 6. Association between IHC expression of CD73 and the histological type of tumor in malignant cases.

Histological Type	Score								P value
	score0		score+1		score+2		score+3		
	N	%	N	%	N	%	N	%	
IDC	3	100	4	50.00	10	52.63	11	73.33	0.422 NS
ILC	0	0.00	0	0.00	4	21.05	0	0.00	
DCIS	0	0.00	0	0.00	1	5.26	0	0.00	
IPC	0	0.00	1	12.50	1	5.26	0	0.00	
IDC & DCIS	0	0.00	1	12.50	3	15.79	2	13.33	
IDC & ILC	0	0.00	2	25.00	0	0.00	2	13.33	
Total	3	100%	8	100%	19	100%	15	100%	
Statistically significant differences between percentages using the Pearson Chi-square test at the 0.05 level. * (significant), ** (high significant) and NS: Non-Significantly.									

TABLE 7. Association between IHC expression of CD73 and the histological type of tumor in benign cases.

Histological Type	Score of CD73								P value
	Score 0		Score +1		Score +2		Score +3		
	N	%	N	%	N	%	N	%	
FCC	4	28.57	1	6.25	3	33.33	0	0.00	0.015*
Fibroadenoma	4	28.57	1	6.25	0	0.00	1	20.00	
Fibroadenoma & FCC	1	7.14	3	18.75	1	11.11	1	20.00	
FCC & FAC	2	14.29	0	0.00	0	0.00	0	0.00	
Phyllodes & FCC	1	7.14	0	0.00	0	0.00	0	0.00	
GM&FAC	1	7.14	0	0.00	0	0.00	0	0.00	
Fibroadenoma & ADH with atypia	0	0.00	0	0.00	0	0.00	1	20.00	
Intraductal papilloma & ADH	0	0.00	0	0.00	0	0.00	1	20.00	
FCC & chronic granulomatous mastitis	1	7.14	0	0.00	0	0.00	0	0.00	
FCC & hyperplasia without atipea	0	0.00	0	0.00	2	22.22	1	20.00	
Fibroadenoma & hyperplastic	0	0.00	0	0.00	1	11.11	0	00.00	
Gynecomastia & Ductalhyperplasia	0	0.00	2	12.50	0	0.00	0	0.00	
Gynecomastia&FCC	0	0.00	5	31.25	0	0.00	0	0.00	
Ductalhyperplasia	0	0.00	2	12.50	1	11.11	0	0.00	
Gynecomastia	0	0.00	2	12.50	1	11.11	0	0.00	
Statistically significant differences between percentages using the Pearson Chi-square test at the 0.05 level. * (significant), ** (high significant) and NS: Non-Significantly.									

6- Association of IHC expression of CD73 with lymph nodes involvement

There was insignificant relation (P= 0.728) between CD73 expression and lymph node involvement in malignant group Table 8.

TABLE 8. Association between IHC expression of CD73 and the Lymph node Metastasis

Lymph node Metastasis	Score of CD73								P-value
	score 0		score +1		Score +2		score +3		
	N	%	N	%	N	%	N	%	
Positive	2	66.67	4	66.67	11	21.43	8	88.89	0.728 NS
Negative	1	33.33	2	33.33	3	78.57	1	11.11	
Statistically significant differences between percentages using the Pearson Chi-square test at the 0.05 level * (significant), ** (high significant) and NS: Non-Significantly.									

*It is worth noting that there are 13 cases in which it is unknown whether there are metastases in lymph nodes or not.

7- Association of IHC expression of CD73 with histological grade

This study revealed insignificant association (P=0.054) between CD73 positive expression and tumor grade in malignant group Table 9.

TABLE 9. Association of IHC expression of CD73 with tumor grade in malignant group

Score of CD73	Tumor grade					
	I		II		III	
	N	%	N	%	N	%
Score 0	0	0.00	3	10.34	0	0.00
Score +1	1	50.00	6	20.69	1	7.14
Score +2	0	0.00	15	51.72	4	28.57
Score +3	1	50.00	5	17.24	9	64.29
P value	0.054 NS					
Statistically significant differences between percentages using the Pearson Chi-square test at the 0.05 level. * (significant), ** (high significant) and NS: Non-Significantly.						

8- Association of IHC expression of CD73 with pathological stage

This study revealed insignificant association (P=0.824) between CD73 positive expression and pathological stage in malignant group Table 10.

TABLE 10. Association between IHC expressions of CD73 with pathological stage

Score of CD73	Tumor stage											
	I		II				III					
	IA		IIA		IIB		IIIA		IIIB		IIIC	
	N	%	N	%	N	%	N	%	N	%	N	%
Score 0	0	0.00	1	14.29	0	0.00	2	25.00	0	0.00	0	0.00
Score +1	0	0.00	1	14.29	2	40.00	2	25.00	0	0.00	1	11.11
Score +2	1	100	4	57.14	2	40.00	2	25.00	1	50.00	4	44.44
Score +3	0	0.00	1	14.29	1	20.00	2	25.00	1	50.00	4	44.44
P-value	0.824 NS											
Statistically significant differences between percentages using the Pearson Chi-square test at the 0.05 level * (significant), ** (high significant) and NS: Non-Significantly.												

*It is worth noting that there are 13 cases whose stage is unknown.

9- Association of IHC expression of CD73 with ER expression

This study revealed highly significant differences ($P < 0.001$) between the expression of CD73 and ER expression (positive and negative) in malignant cases; the highest CD73 expression was seen in negative ER cases, comprising 86.67% Table 11.

TABLE 11. Association between IHC expression of CD73 and ER expression

ER Expression	Score of CD73								P-value
	score 0		score +1		Score +2		score +3		
	N	%	N	%	N	%	N	%	
Positive	3	100.00	5	83.33	11	78.57	2	13.33	
Negative	0	0.00	1	16.67	3	21.43	13	86.67	< 0.001**
Statistically significant differences between percentages using the Pearson Chi-square test at the 0.05 level * (significant), ** (high significant) and NS: Non-Significantly.									

*It is worth noting that there are 7 cases whose ER expression is unknown.

10- Association of IHC expression of CD73 with PR expression

This study revealed highly significant differences (P = 0.003) between the expression of CD73 and PR expression (positive and negative) in malignant cases; the highest CD73 expression was detected in negative PR cases, comprising 86.67% Table 12.

TABLE 12. Association between IHC expression of CD73 and PR expression

PR Expression	Score of CD73								P-value
	score 0		score +1		Score +2		score +3		
	N	%	N	%	N	%	N	%	
Positive	2	66.67	4	60.00	11	76.92	2	13.33	0.003**
Negative	1	33.33	2	40.00	3	23.08	13	86.67	
Statistically significant differences between percentages using the Pearson Chi-square test at the 0.05 level. * (significant), ** (high significant) and NS: Non-Significantly.									

*It is worth noting that there are 7 cases whose PR expression is unknown.

11- Association of IHC expression of CD73 with HER2/neu expression

This study revealed significant differences (P = 0.041) between the expression of CD73 and HER2/neu (positive and negative) in malignant cases; the highest CD73 expression (score 3) was seen in negative HER2/neu cases, comprising 93.33% Table 13.

TABLE 13. Association between IHC expressions of CD73 with HER2/neu expression

HER2/neu Expression	Score of CD73								P-value
	score 0		score +1		Score +2		score +3		
	N	%	N	%	N	%	N	%	
Positive	0	0.00	2	33.33	7	50.00	1	6.67	
Negative	3	100.00	4	66.67	7	50.00	14	93.33	0.041*
Statistically significant differences between percentages using the Pearson Chi-square test at the 0.05 level. * (significant), ** (high significant) and NS: Non-Significantly.									

*It is worth noting that there are 7 cases whose HER2/neu expression is unknown.

12- Association of IHC expression of CD73 with molecular subtypes

This study revealed highly significant differences ($P < 0.001$) between the expression of CD73 and molecular subtypes in malignant cases, the highest CD73 expression was seen in triple-negative cases, comprising 92.3% Table 14.

TABLE 14. Association between IHC expressions of CD73 with molecular subtypes

Score of CD73	Molecular subtypes					
	Luminal		HER-2 positive		Triple negative	
	N	%	N	%	N	%
Score 0	3	14.3	0	0.00	0	0.00
score +1	5	23.8	1	25.00	0	0.00
score +2	11	52.4	2	50.00	1	7.7
score +3	2	9.5	1	25.00	12	92.3
P value	< 0.001**					
Statistically significant differences between percentages using the Pearson Chi-square test at the 0.05 level * (significant), ** (high significant) and NS: Non-Significantly.						

*It is worth noting that there are 7 cases whose molecular subtype is unknown.

DISCUSSION

To the best of our knowledge, this is the first study in Iraq to look at the expression of the CD73 ectoenzyme in BC using IHC and examine the relationship between the expression of this marker and several clinicopathological characteristics.

The current study found that CD73 was expressed positively in most malignant cases, accounting for approximately 75.56% of all cases. Additionally, there were significant correlations between breast tumor type and CD73 expression ($P < 0.001$). This outcome comes to an agreement with Ismail and Yousif, (2021), who stated in their study that CD73 levels in BC patients were significantly higher compared with control[17]. According to the recent researches, CD73 is overexpressed in a variety of solid malignant tumors such as breast cancer, ovarian cancer, gallbladder cancer, colorectal cancer, and prostate cancer[18]. CD73 is involved in a variety of cancer-related activities The relationship between CD73 overexpression and cancer subtype, prognosis, and drug response in patients has established CD73's potential as a detectable biomarker in approaching cancer therapy[19]. Many studies have discovered a link between increased CD73 expression and a worse prognosis in cancer patients[20]. Wang et al. (2008) discovered that CD73, through its enzymatic activity of generating adenosine, may increase human breast cancer cell adhesion, migration, and invasion[21]. The function of CD73 increases in cancer when ATP and AMP are released into the extracellular space. In this case, CD73 interacts with fibronectin and laminin, promoting cancer cell motility and metastasis in human BC cells. CD73 is also widely expressed in the cells that comprise the BC tumor microenvironment, to the extent that overexpression of CD73 in cancer cells has been linked to poor overall survival in BC patients. Hypoxia promotes angiogenesis and a high rate of cell proliferation, both of which enhance hypoxia-inducible factor (HIF-1) overexpression in the tumor microenvironment. As a result, purinergic pathway genes such as CD73 and CD39 are positively regulated[22].

Regarding the type of tumor, the malignant cases showed the highest positive expression of CD73 in 75.56% of total, which is compatible with Jiang et al. (2018) who stated that CD73 was expressed in 74.30% of malignant tumors[23]. For benign tumors, negative CD73 expression was seen in 68.18% of cases, this comes in consistency with a study by Soliman and Mohamad, 2022 who stated that CD73 immunoreactivity was only in 12.5% of cases in benign colorectal tumors and the expression levels were lower than in malignant tumor specimens[24]. Moreover, the study results showed negative CD73 expression in 100% of normal breast duct tissues. Monteiro et al. (2021) found on his work in other type of tumor that most medullary carcinomas and non-neoplastic para follicular C-cells were CD73-negative, demonstrating that CD73 expression is absent in both normal and cancerous tissues[25].

Other aspect in this study is the association of CD73 expression with some clinicopathological parameters, specifically age, which was the highest in malignant group patients by age of 40–49 comprising 63.6%. While for the patient group of 50–59 years old, the expression of this marker was seen in 41.2%, and there were no significant differences between CD73 expression and age for malignant cases compared to the benign group. A study by YU et al. (2017) found no significant correlation between CD73 expression and age in BC, which is consistent with the findings of this study[26]. Also, in a study about CD73 expression in TNBC, there were no significant differences between CD73 expression and age[27]. Another study on gastric cancer patients showed there was no significant association between CD73 expression and age[28]. While a significant relationship between CD73 expression and age was shown in a study of colorectal carcinoma patients[14].

Aging is described as a progressive decline of the immune system[29]. The thymus, a major lymphoid organ where T lymphocytes grow, decreases and replaced by adipose tissue as we age. As a result, the number of naive T cells exiting the thymus slowly diminishes. This mechanism is particularly visible in CD8+ T cells. With increasing in age, there is a relative increase in functionally exhausted memory and effector cells due to the decreased output of naive T lymphocytes[30]. Adenosine, as known as a result of AMP hydrolyzing by CD39 and CD73, which is known to inhibit the function of CD4+ and CD8+ T cells as well as the lytic activity of NK cells, the effect of aging on T-cell tumors Infiltrating lymphocytes via decreased cytokine production, increased apoptosis, and decreased lymphocyte association[31].

In this study, moreover, the correlation between tumor site and diameter for both malignant and benign BC also revealed no significant differences with CD73 expression. These findings are consistent with the findings of YU et al. (2017), who found no correlation between CD73 expression and tumor diameter in BC[26]. Conversely, in other studies of BC patients, it was discovered that tumor-infiltrating NK cells elevated CD73 expression and that the frequency of these CD73-positive NK cells was associated with larger breast tumors[13]. As pointed out by Azambuja et al. (2019), in their study, ADO accumulation favors tumor growth, metastasis, angiogenesis, chemo-resistance, and immunosuppression. They used small interfering RNA (siRNA) technology in their study to inhibit CD73 and prevent the positive actions of CD73 on tumor growth in glioblastoma, where observed tumor size was reduced by 45 and 40%, respectively. This was followed by a 95% decline in ADO levels in cerebrospinal fluid, confirming the function of extracellular ADO in glioma growth in vivo[32].

In this study, a highly significant correlation was observed between CD73 expression and gender within benign tumors. Despite females had the most expression, accounting for 23.33% of score 2 and 16.67% of score 3, while males only had 14.29% of score 2, but there was no significant association between CD73 expression and the gender of malignant cases. A study by Ranjbar et al. (2019) showed there was no significant association between CD73 expression and gender with salivary gland tumors[33]. In another study, there was no association between CD73 and the gender of the tumor in gastric cancer[28]. At the most fundamental level, most in vivo CD73 studies have been conducted in male mice or when biological sex was not explicitly considered as a potential variable[34].A novel notion in the area is that key hormonal factors, particularly estrogen-derived impacts, alter how males and females metabolize extracellular adenosine and deal with CD73 deficiency. Differences in adenosine signaling play a role in neuromodulation inside the hippocampus, which has a high frequency of spontaneous transient adenosine events that alter synaptic transmission, glia-neuro connections, and other key processes[18]. Female mice appear to rely more on CD73 for spontaneous adenosine transients, which is consistent with recent results that CD73 expression and activity in the hippocampus are favorably regulated by estrogen[35].

This study revealed no significant association between CD73 expression and the histological type of malignant cases although the higher expression of malignant cases was recorded in the IDC comprising 73.33% of score 3. However, there was a relationship between the expression of CD73 and the histological type of benign cases.

This result agrees with Supernat et al. (2012), who showed no correlation was found between CD73 expression and the histological type of BC[36]. Another study found no relationship between CD73 and histology type in renal cell carcinoma[37]. While Choi et al., 2020 showed in their results high significant correlations between histological type and CD73 expression in BC in which the expression in IDC was significantly higher than that in DCIS13. Upregulation of CD73 enhanced cell migration and invasion in pcDNA-NT5E transfected BC T-47D cells, and CD73 overexpression increased cell mobility[38]. CD73's involvement in tumor progression is also linked to its protein structure, which regulates cell-cell and cell-extracellular matrix adhesion[39]. Overexpression of CD73 in Hela and SiHa cells, for example, increased proliferation and migration irrespective of its enzymatic activity, as high concentrations of adenosine reduced these effects, while adenosine 5'-(α,β-methylene)diphosphate (APCP) blockade of the enzyme did not reverse the impact[40]. As a result, this molecule is important in cell adhesion, motility, tumor cell-extracellular matrix interactions, and metastasis[41].

The present study found no significant link between CD73 expression and lymph node involvement. These findings are consistent with those of Zhou et al. (2019), who found no correlation between CD73 expression and lymph node involvement in pancreatic cancer[42]. A previous study has linked CD73 expression to enhanced breast invasion, migration, and lymph node metastases[43]. Tumor cells use a variety of strategies to avoid immune monitoring during tumor development and metastasis, including alterations in adenosine signaling. CD73 is essential for catalyzing the hydrolysis of AMP into adenosine. CD73-generated adenosine can bind to four different G-protein-coupled receptors to mediate its immunosuppressive function: A1R, A2A R, A2B R, and A3R, exerting its effect on the immune system via various pathways[44]. They also revealed that CD73 could increase head and neck squamous cell carcinoma migration and invasion via adenosine A3R stimulation[44]. This could be one of the mechanisms behind the link between CD73 expression and lymph node metastasis. A growing body of evidence suggests that the CD73-adenosine pathway is important in cancer development and immune evasion[23]. Adenosine produced by CD73 may contribute to the establishment of an immunosuppressive environment by decreasing the anti-tumor activity of immune cells such as CD8+ positive T cells and NK cells[45].

The current study showed no significant association between CD73 with stage and grade. Kruger et al. (1991) revealed that there was no significant association between CD73 expression and tumor (stage and grade of patients') in BC[46]. Zhou et al. (2019) found no significant association between CD73 expressions with grade, but there was a relationship with stage[42]. CD73 overexpression has been linked to a poor clinical outcome in patients with high-grade serous ovarian cancer, colorectal cancer, non-small cell lung cancer, gastric cancer, oral squamous cell carcinoma, and triple-negative BC[47–49]. Mei et al. (2020) found that positive CD73 expression was more common in renal cell carcinoma patients with grades 3–4, stages III–IV, and lymph node metastasis, implying that CD73 expression is also linked to tumor aggressiveness, invasion ability, and metastasis in renal cell carcinoma. Adenosine, which is abundant in the tumor microenvironment, plays an important role in anti-tumor immunosuppression. CD73 is essential for adenosine synthesis and has immunosuppressive activities. Other critical contributors to the elevation of CD73 expression in tumor tissues, in addition to hypoxia and adenosine-rich environments, are high levels of transforming growth factor β (TGFβ), interferon, and tumor necrosis factor (TNFα) in the tumor microenvironment[50].

Moreover, in this task the findings revealed a highly significant ($P < 0.001$) association between CD73 positive expression and ER, where the highest expression of CD73 (86.67%) score 3 was in ER-negative cases, while in ER positive cases there was 78.57% of cases in score 2. As proven by Katsuta et al., 2018, Katsuta et al., 2019, the level of CD73 expression in ER (+) breast malignancies is significantly lower than in ER (-) tumors[51,52]. Spychala et al., 2004, found ER-negative cells have highly expressed CD73 protein and mRNA and produce up to 104-fold more adenosine from AMP and ATP[53].

In a study about the role of ER in the regulation of CD73 and adenosine in BC, The mechanism of ER-mediated gene regulation may be direct, i.e., through binding to the ER-responsive element, or it can function indirectly by binding to transcription factors or proteins that form multi-protein complexes inside the transcription machinery or by influencing the expression of other transcription factors[54]. Another study discovered that because the ER consensus binding site was not detected within the CD73 promoter at its complete length (969 base pairs), the down-regulation of CD73 expression by ER is most likely an indirect process. The same study discovered that estrogen, acting through the ER, significantly reduces CD73 expression. CD73's major role is to create extracellular adenosine, and given its well-defined antigenic, cytoprotective, and anti-inflammatory properties, the researchers hypothesized that enhanced adenosine synthesis in ER-negative BC could have direct tumor-promoting consequences[53].

Triple negative breast cancer (TNBC) accounts for 10% to 15% of all incidences of breast cancer. TNBCs do not react to hormonal or anti-HER2 therapy because they lack estrogen and progesterone receptors and express low amounts of HER2. TNBC is a very aggressive form of breast cancer with a poor prognosis when compared to other BC subtypes[55]. This study revealed a significant association between CD73 positive expression and negative hormone receptor status for ER, PR, and HER2 negative, as well as showed a highly significant ($P < 0.001$) correlation with molecular subtypes. Where overexpression of CD73 was found in triple negative compared with other subtypes. These findings are consistent with those of a study conducted by YU et al. (2017) who proved that there was a significant association between CD73 expression and ER, PR, and HER2 negative expressions[26]. While Choi et al., 2020, discovered no significant relationship between CD73 and ER, HER2, or molecular subtypes, but they did find a correlation with positive PR[13]. Although, Qiao et al. (2019) found a relation between CD73 and molecular subtypes, their study revealed that the protein's expression was higher in TNBC samples than in the other two subtypes[56].In general, high expression of CD73 is associated with worse clinical outcomes in cancer patients[19]. However, in BC, the predictive importance of CD73 is still controversial36. Furthermore, several studies have shown that CD73 has predictive relevance in TNBC[47,57].

In a meta-analysis study of all BC subtypes, elevated levels of CD73 mRNA data were found to be substantially linked with worse overall survival (OS) and enhanced anthra-cycline resistance in the TNBC subtype[47]. Several studies also found the high expression of CD73 in BC was significantly correlated with poor overall survival in TNBC[23,48,56]. Petruk et al. (2021) investigated the probable cellular mechanisms by which CD73 may promote TNBC in addition to its immunosuppressive effect[58]. Their findings revealed that suppressing CD73 expression and activity in normoxia decreases TNBC viability, proliferation, and migration in vitro. When tumors grow larger than 1 cm, hypoxia usually develops[59]. Furthermore, hypoxia is one of the elements that increases ATP synthesis, which is quickly converted to adenosine, resulting in an anti-inflammatory response[60]. Their findings showed Petruk et al. (2021) that the inhibitory effects of CD73 deficiency on cell viability were hypoxia-dependent. In addition, they discovered that suppressing CD73 decreases cellular protrusion elongation in both normoxia and hypoxia[58]. In a study involving CD73 expression and NT5E CpG island methylation, researchers showed that CD73 expression is regulated in breast cancer by the methylation status of the CpG Island. Methylation was inversely linked with CD73 expression in BC cell lines and primary BC. The findings also reveal that methylation-dependent transcriptional silencing reduces expression in ER-positive (predominantly luminal) BC cell lines, whereas in ER-negative and triple-negative cell lines, CD73 is often overexpressed. The absence of methylation in CD73 in ER-negative and TNBC suggests that inhibiting CD73 may be an appealing treatment strategy in these breast cancer subgroups[61]. In another study by Jeong et al., 2020, it was also discovered that methylation of the NT5E gene was related to breast cancer development and was associated with poor prognostic factors in BC20.

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Furthermore, ER-negative and TNBC-positive breast cancer patients with un-methylated NT5E have lower disease-free survival and overall survival than ER-positive BC patients. NT5E methylation was found to be inversely related to TP53 mutation status while being favorably connected to ADO signaling[62].

CONCLUSION

The positive expression of CD73 ectonucleotidase was found in most malignant cases, but it was negative in most of benign samples, although some benign cases showed overexpression of CD73, while no expression was seen in normal ductal breast tissue samples. A significant association was found between CD73 with gender and histological types in benign cases, and with (ER, PR, HER-2) negative and triple negative molecular subtypes in malignant cases. This allowed us to suggest that inhibiting CD73 could be an appealing treatment strategy in the TNBC subgroup.

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REFERENCES

1. Kolak A, Kamińska M, Sygit K, Budny A, Surdyka D, Kukiełka-Budny B, et al. Primary and secondary prevention of breast cancer. Ann Agric Environ Med. 2017;24:549–53. doi:10.26444/aaem/75943
2. Alwan NAS, Tawfeeq FN, Mallah NAG. Demographic and clinical profiles of female patients diagnosed with breast cancer in Iraq. J Contemp Med Sci. 2019;5:14–9. doi:10.22317/jcms.v5i1.544
3. Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, et al. Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes Dis. 2018;5:77–106. doi:10.1016/j.gendis.2018.05.001
4. Hafez A, Mohamed H. Assessment of the Perception of Aesthetics and Smile Attractiveness among Dental and Health Students. Int J Oncol Res [Internet]. 2022 [cited 2022 Nov 16];5. Available from: https://clinmedjournals.org/articles/ijor/international-journal-of-oncology-research-ijor-5-037.php?jid=ijor doi:10.23937/2643-4563/1710037
5. Hussein TA, Tamemi IAA. Micro RNA 145 as Biomarker for Breast cancer. Res J Pharm Technol. 2019;12:5923. doi: 10.5958/0974-360X.2019.01027.8.
6. Suhail Y, Cain MP, Vanaja K, Kurywchak PA, Levchenko A, Kalluri R, et al. Systems Biology of Cancer Metastasis. Cell Syst. 2019;9:109–27. doi:10.1016/j.cels.2019.07.003
7. Alrawi N. A review on breast cancer in Iraq and future therapies insights. Baghdad J Biochem Appl Biol Sci. 2022;3:4–16. doi:10.47419/bjbabs.v3i01.64
8. Green AR, Powe DG, Rakha EA, Soria D, Lemetre C, Nolan CC, et al. Identification of key clinical phenotypes of breast cancer using a reduced panel of protein biomarkers. Br J Cancer. 2013;109:1886–94. doi:10.1038/bjc.2013.528
9. Jayashree V, Velraj M. Breast Cancer and various Prognostic Biomarkers for the diagnosis of the disease: A Review. Res J Pharm Technol. 2017;10:3211. doi: 10.5958/0974-360X.2017.00570.4.
10. Iwamoto T, Kajiwara Y, Zhu Y, Iha S. Biomarkers of neoadjuvant/adjuvant chemotherapy for breast cancer. Chin Clin Oncol. 2020;9:27–27. doi:10.21037/cco.2020.01.06
11. Fisusi FA, Akala EO. Drug Combinations in Breast Cancer Therapy. Pharm Nanotechnol. 2019;7:3–23. doi:10.2174/2211738507666190122111224
12. Allard B, Allard D, Buisseret L, Stagg J. The adenosine pathway in immuno-oncology. Nat Rev Clin Oncol. 2020;17:611–29. doi:10.1038/s41571-020-0382-2
13. Choi HR, Oh HK, Park SH, Jeong Y. Expression of CD73 is associated with tumor progression and intratumoral inflammation in breast cancer. Asia Pac J Clin Oncol. 2022;18:35–43. doi:10.1111/ajco.13450
14. Messaoudi N, Cousineau I, Arslanian E, Henault D, Stephen D, Vandenbroucke-Menu F, et al. Prognostic value of CD73 expression in resected colorectal cancer liver metastasis. OncoImmunology. 2020;9:1746138. doi:10.1080/2162402X.2020.1746138
15. Rocha P, Salazar R, Zhang J, Ledesma D, Solorzano JL, Mino B, et al. CD73 expression defines immune, molecular, and clinicopathological subgroups of lung adenocarcinoma. Cancer Immunol Immunother. 2021;70:1965–76. doi:10.1007/s00262-020-02820-4

16. Magaki S, Hojat SA, Wei B, So A, Yong WH. An Introduction to the Performance of Immunohistochemistry. In: Yong WH, editor. Biobanking [Internet]. New York, NY: Springer New York; 2019 [cited 2022 Nov 16]. p. 289–98. Available from: http://link.springer.com/10.1007/978-1-4939-8935-5_25 doi:10.1007/978-1-4939-8935-5_25
17. Ismail PA, Yousif AM. Diagnosis of Breast Cancer by some remarkable enzymes. Iraq Med J. 2021;5:101–3. doi:10.22317/imj.v5i3.1056
18. Wang R, Zhang Y, Lin X, Gao Y, Zhu Y. Prognositic value of CD73-adenosinergic pathway in solid tumor: A meta-analysis and systematic review. Oncotarget. 2017;8:57327–36. doi:10.18632/oncotarget.16905
19. Gao Z, Dong K, Zhang H. The Roles of CD73 in Cancer. BioMed Res Int. 2014;2014:1–9. doi:10.1155/2014/460654
20. Jeong YJ, Oh HK, Choi HR, Park SH. Methylation of the NT5E Gene Is Associated with Poor Prognostic Factors in Breast Cancer. Diagnostics. 2020;10:939. doi:10.3390/diagnostics10110939
21. Wang L, Zhou X, Zhou T, Ma D, Chen S, Zhi X, et al. Ecto-5'-nucleotidase promotes invasion, migration and adhesion of human breast cancer cells. J Cancer Res Clin Oncol. 2008;134:365–72. doi:10.1007/s00432-007-0292-z
22. de Araújo JB, Kerkhoff VV, de Oliveira Maciel SFV, de Resende E Silva DT. Targeting the purinergic pathway in breast cancer and its therapeutic applications. Purinergic Signal. 2021;17:179–200. doi:10.1007/s11302-020-09760-9
23. Jiang T, Xu X, Qiao M, Li X, Zhao C, Zhou F, et al. Comprehensive evaluation of NT5E/CD73 expression and its prognostic significance in distinct types of cancers. BMC Cancer. 2018;18:267. doi:10.1186/s12885-018-4073-7
24. Falih Soliman N, Jasim Mohamad B. The Impact of CD37 Ectoenzyme Expression in Benign and Malignant Colorectal Tumors. Arch Razi Inst. Razi Vaccine & Serum Research Institute; 2022;77:2049–57. doi:10.22092/ari.2022.358611.2261
25. Monteiro I, Missiaglia E, Sciarra A, Santos JV, Bouilly J, Romero P, et al. CD73 expression in normal, hyperplastic, and neoplastic thyroid: a systematic evaluation revealing CD73 overexpression as a feature of papillary carcinomas. Virchows Arch. 2021;479:209–14. doi:10.1007/s00428-021-03100-x
26. YU K, NI C, SONG X, YUAN H, SUN K. Expression of CD73 in breast cancer and the correlation with its clinicopathological features. Chin J Clin Pharmacol Ther. 2017;22:551.
27. Cerbelli B, Botticelli A, Pisano A, Pernazza A, Campagna D, De Luca A, et al. CD73 expression and pathologic response to neoadjuvant chemotherapy in triple negative breast cancer. Virchows Arch. 2020;476:569–76. doi:10.1007/s00428-019-02722-6
28. Hu S, Meng F, Yin X, Cao C, Zhang G. NT5E is associated with unfavorable prognosis and regulates cell proliferation and motility in gastric cancer. Biosci Rep. 2019;39:BSR20190101. doi:10.1042/BSR20190101
29. Montecino-Rodriguez E, Berent-Maoz B, Dorshkind K. Causes, consequences, and reversal of immune system aging. J Clin Invest. 2013;123:958–65. doi:10.1172/JCI64096
30. Jeske SS, Schuler PJ, Doescher J, Theodoraki MN, Laban S, Brunner C, et al. Age-related changes in T lymphocytes of patients with head and neck squamous cell carcinoma. Immun Ageing. 2020;17:3. doi:10.1186/s12979-020-0174-7
31. Häusler SFM, Montalbán del Barrio I, Strohschein J, Anoop Chandran P, Engel JB, Hönig A, et al. Ectonucleotidases CD39 and CD73 on OvCA cells are potent adenosine-generating enzymes responsible for adenosine receptor 2A-dependent suppression of T cell function and NK cell cytotoxicity. Cancer Immunol Immunother. 2011;60:1405–18. doi:10.1007/s00262-011-1040-4
32. Azambuja JH, Gelsleichter NE, Beckenkamp LR, Iser IC, Fernandes MC, Figueiró F, et al. CD73 Downregulation Decreases In Vitro and In Vivo Glioblastoma Growth. Mol Neurobiol. 2019;56:3260–79. doi:10.1007/s12035-018-1240-4
33. Ranjbar M, Ranjbar Z, Zahed M, Nikookar N. CD73 a novel marker for the diagnosis of benign and malignant salivary gland tumors. J Clin Exp Dent. 2019;0–0. doi:10.4317/jced.54918
34. Alcedo KP, Bowser JL, Snider NT. The elegant complexity of mammalian ecto-5′-nucleotidase (CD73). Trends Cell Biol. 2021;31:829–42. doi:10.1016/j.tcb.2021.05.008
35. Mitrović N, Dragić M, Zarić M, Drakulić D, Nedeljković N, Grković I. Estrogen receptors modulate ectonucleotidases activity in hippocampal synaptosomes of male rats. Neurosci Lett. 2019;712:134474. doi:10.1016/j.neulet.2019.134474
36. Supernat A, Markiewicz A, Wełnicka-Jaśkiewicz M, Seroczyńska B, Skokowski J, Sejda A, et al. CD73 Expression as a Potential Marker of Good Prognosis in Breast Carcinoma. Appl Immunohistochem Mol Morphol. 2012;20:103–7. doi:10.1097/PAI.0b013e3182311d82
37. Tripathi A, Lin E, Xie W, Flaifel A, Steinharter JA, Stern Gatof EN, et al. Prognostic significance and immune correlates of CD73 expression in renal cell carcinoma. J Immunother Cancer. 2020;8:e001467. doi:10.1136/jitc-2020-001467

38. Zhou X, Zhi X, Zhou P, Chen S, Zhao F, Shao Z, et al. Effects of ecto-5'-nucleotidase on human breast cancer cell growth in vitro and in vivo. Oncol Rep. 2007;17:1341–6.
39. Iser IC, Andrade Mello P, Davies S, Souza Santos JF, Pilger DA, Buffon A, et al. A three-dimensional microenvironment alters CD73 expression in cervical cancer. Cell Biochem Funct. 2021;39:780–90. doi:10.1002/cbf.3649
40. Gao Z, Wang H, Lin F, Wang X, Long M, Zhang H, et al. CD73 promotes proliferation and migration of human cervical cancer cells independent of its enzyme activity. BMC Cancer. 2017;17:135. doi:10.1186/s12885-017-3128-5
41. Katsuta E, Tanaka S, Mogushi K, Shimada S, Akiyama Y, Aihara A, et al. CD73 as a therapeutic target for pancreatic neuroendocrine tumor stem cells. Int J Oncol. 2016;48:657–69. doi:10.3892/ijo.2015.3299
42. Zhou L, Jia S, Chen Y, Wang W, Wu Z, Yu W, et al. The distinct role of CD73 in the progression of pancreatic cancer. J Mol Med Berl Ger. 2019;97:803–15. doi:10.1007/s00109-018-01742-0
43. Zhi X, Wang Y, Yu J, Yu J, Zhang L, Yin L, et al. Potential prognostic biomarker CD73 regulates epidermal growth factor receptor expression in human breast cancer. IUBMB Life. 2012;64:911–20. doi:10.1002/iub.1086
44. Ren Z-H, Lin C-Z, Cao W, Yang R, Lu W, Liu Z-Q, et al. CD73 is associated with poor prognosis in HNSCC. Oncotarget. 2016;7:61690–702. doi:10.18632/oncotarget.11435
45. Cekic C, Linden J. Adenosine A2A Receptors Intrinsically Regulate CD8+ T Cells in the Tumor Microenvironment. Cancer Res. 2014;74:7239–49. doi:10.1158/0008-5472.CAN-13-3581
46. Krüger K, Thompson L, Kaufmann M, Möller P. Expression of ecto-5'-nucleotidase (CD73) in normal mammary gland and in breast carcinoma. Br J Cancer. 1991;63:114–8. doi:10.1038/bjc.1991.23
47. Loi S, Pommey S, Haibe-Kains B, Beavis PA, Darcy PK, Smyth MJ, et al. CD73 promotes anthracycline resistance and poor prognosis in triple negative breast cancer. Proc Natl Acad Sci. 2013;110:11091–6. doi:10.1073/pnas.1222251110
48. Buisseret L, Pommey S, Allard B, Garaud S, Bergeron M, Cousineau I, et al. Clinical significance of CD73 in triple-negative breast cancer: multiplex analysis of a phase III clinical trial. Ann Oncol. 2018;29:1056–62. doi:10.1093/annonc/mdx730
49. Turcotte M, Spring K, Pommey S, Chouinard G, Cousineau I, George J, et al. CD73 Is Associated with Poor Prognosis in High-Grade Serous Ovarian Cancer. Cancer Res. 2015;75:4494–503. doi:10.1158/0008-5472.CAN-14-3569
50. Mei X, Shu J, Huang R, Chu X, Tian Y. Expression of VEGF, CD73 and their relationship with clinical pathology, microvessel density, and prognosis in renal cell carcinoma. Transl Androl Urol. 2020;9:1366–73. doi:10.21037/tau-20-904
51. Katsuta E, Dasgupta S, Yan L, Takabe K. Higher CD73 Expression Is Associated with Poor Prognosis in Estrogen Receptor-Positive Breast Cancer. J Am Coll Surg. 2018;227:S34. doi:10.1016/j.jamcollsurg.2018.07.054
52. Katsuta E, Anand V, Yan L, Dasgupta S, Takabe K. Abstract 5200: High CD73 expression, regulated by estrogen signaling, associates with cancer aggressiveness in estrogen receptor (+) breast cancer. Cancer Res. 2019;79:5200–5200. doi:10.1158/1538-7445.AM2019-5200
53. Spychala J, Lazarowski E, Ostapkowicz A, Ayscue LH, Jin A, Mitchell BS. Role of Estrogen Receptor in the Regulation of Ecto-5′-Nucleotidase and Adenosine in Breast Cancer. Clin Cancer Res. 2004;10:708–17. doi:10.1158/1078-0432.CCR-0811-03
54. Zelinski DP, Zantek ND, Walker-Daniels J, Peters MA, Taparowsky EJ, Kinch MS. Estrogen and Myc negatively regulate expression of the EphA2 tyrosine kinase. J Cell Biochem. 2002;85:714–20. doi:10.1002/jcb.10186
55. Won K, Spruck C. Triple-negative breast cancer therapy: Current and future perspectives (Review). Int J Oncol. 2020;57:1245–61. doi:10.3892/ijo.2020.5135
56. Qiao Z, Li X, Kang N, Yang Y, Chen C, Wu T, et al. A Novel Specific Anti-CD73 Antibody Inhibits Triple-Negative Breast Cancer Cell Motility by Regulating Autophagy. Int J Mol Sci. 2019;20:1057. doi:10.3390/ijms20051057
57. Samanta D, Park Y, Ni X, Li H, Zahnow CA, Gabrielson E, et al. Chemotherapy induces enrichment of CD47 + /CD73 + /PDL1 + immune evasive triple-negative breast cancer cells. Proc Natl Acad Sci [Internet]. 2018 [cited 2022 Nov 16];115. Available from: https://pnas.org/doi/full/10.1073/pnas.1718197115 doi:10.1073/pnas.1718197115
58. Petruk N, Tuominen S, Åkerfelt M, Mattsson J, Sandholm J, Nees M, et al. CD73 facilitates EMT progression and promotes lung metastases in triple-negative breast cancer. Sci Rep. 2021;11:6035. doi:10.1038/s41598-021-85379-z
59. Lundgren K, Nordenskjöld B, Landberg G. Hypoxia, Snail and incomplete epithelial–mesenchymal transition in breast cancer. Br J Cancer. 2009;101:1769–81. doi:10.1038/sj.bjc.6605369

60. Boison D, Yegutkin GG. Adenosine Metabolism: Emerging Concepts for Cancer Therapy. Cancer Cell. 2019;36:582–96. doi:10.1016/j.ccell.2019.10.007
61. Lo Nigro C, Monteverde M, Lee S, Lattanzio L, Vivenza D, Comino A, et al. NT5E CpG island methylation is a favourable breast cancer biomarker. Br J Cancer. 2012;107:75–83. doi:10.1038/bjc.2012.212
62. Sidders B, Zhang P, Goodwin K, O'Connor G, Russell DL, Borodovsky A, et al. Adenosine Signaling Is Prognostic for Cancer Outcome and Has Predictive Utility for Immunotherapeutic Response. Clin Cancer Res. 2020;26:2176–87. doi:10.1158/1078-0432.CCR-19-2183

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