

Correlation of CD73 Immunohistochemical Expression and Angiogenesis in Benign and Malignant Salivary Gland Tumors

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Aim: to detect immunohistochemically the expression of CD73 and vascular endothelial receptor-2 (VEGFR-2) in both benign and malignant salivary gland neoplasms and to detect the correlation between CD73 and VEGFR-2 if present.

Materials and methods: The current study consisted of forty specimens of 8 cases normal control group of salivary gland tissue, benign salivary gland (SG) neoplasms including pleomorphic adenoma (PA) and warthin's tumor (WT) and malignant group including mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (ADC), 8 cases for each subgroup. Immunohistochemical staining with anti-CD73 and anti-VEGFR-2 primary antibodies was performed. Immunopositivity area fraction was measured by image analysis software followed by statistical analysis using Student's t-test (unpaired) and ANOVA followed by Tukey. Pearson's correlation was used to correlate between CD73 & VEGFR-2.

Results: Higher expression of CD73 and VEGFR-2 was observed in malignant SG neoplasms than benign neoplasms. In addition, benign neoplasms showed higher expression than normal control group. A significant correlation between CD73 and VEGFR-2 was observed indicating their role as a promising prognostic marker.

Conclusion: The high expression of CD73 and VEGFR-2 indicates poor prognosis and might be used as a reliable prognostic marker in SG neoplasms and there is a direct correlation between them.

Keywords: CD73, VEGFR-2, immune checkpoint, angiogenesis, salivary gland neoplasms.

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Introduction

Salivary glands (SGs) are important organs in our body that are divided into major and minor glands.¹ Salivary glands are formed of ductal and acinar cells, from which a group of tumors may arise either benign or malignant. Pleomorphic adenoma (PA) and Warthin's tumor (WT) are examples of benign tumors whereas malignant tumors include mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (ADC).² The different types of salivary gland neoplasms show a great resemblance histologically and clinically which in turn requires accurate diagnosis.³

Immune check point is a regulator of the immune system which occurs through different ways, one of them is through activating adenosine signaling pathway where adenosine is an immunosuppressive organic compound.⁴ Many studies have demonstrated that neoplasms survive by escaping immunity and immune check point inhibition.⁴

Adenosine production plays a major role in keeping normal hemostasis and preventing tissue damage by excessive inflammatory response.⁵ CD73, which is also known as ecto-5'-nucleotidase, is found in most tissues as a cell surface enzyme, encoded by (NT5E) gene.⁵ CD73 acts as a co-signaling molecule on T lymphocytes and as an adhesion molecule important for lymphocytes binding to endothelium to facilitate trans-endothelial migration.⁶ CD73 is expressed in normal cells such as immune cells as B and T cells, epithelial cells, as well as endothelial cells.⁵ Several cell types in response to inflammatory condition, stress, injury and hypoxia release nucleotides such as adenosine.⁴

CD73 has both enzymatic and non-enzymatic functions. CD73 is an enzyme for adenosine production through converting extracellular adenosine monophosphates (AMP) into adenosine which in turn leads to

immunosuppression.⁶ In addition to the immune regulation function of CD73 through adenosine production, CD73 has non enzymatic functions in tumorigenesis including angiogenesis, metastasis, proliferation and migration.⁷ CD73 enhances proliferation of tumor cells through regulating apoptosis, cell cycle and multiple signaling pathways.⁷

Concerning extracellular adenosine, it stimulates angiogenesis through the release of basic fibroblast growth factor (BFGF) and vascular endothelial growth factor (VEGF) which are pro-angiogenic factors.⁸ Vascular endothelial growth factor receptor 2 (VEGFR-2) is one of the important factors involved in angiogenesis where new blood vessels are formed.⁹ VEGFR-2 is known to initiate neoplastic angiogenic process through recruitment and proliferation of the endothelial cells.⁹

Little research has been conducted on the diagnostic and prognostic value of CD73 in oral and maxillofacial tumors. Ren et al¹⁰ showed that CD73 was a promising prognostic marker for oral squamous cell carcinoma. However, there are few studies available concerning the predictive ability of CD73 expression in salivary gland neoplasms.¹⁰

Material and methods

Sample size calculation

A power analysis was designed to have adequate power to apply a statistical test on the null hypothesis that there was no difference found between different tested groups regarding level of the immunohistochemical markers. Through adopting alpha level of (0.05), beta of (0.2) i.e. power =80% and an effect size (f) of (0.584) calculated based on the results of a previous study; the predicted sample size (n) was a total of (40) samples divided into 3 main groups. The first group included a control group which consists of (8) cases. The second group of benign SG neoplasms is

divided into 2 subgroups and each subgroup consists of (8) cases. The third group of malignant SG neoplasms is divided into 2 subgroups and each subgroup consists of (8) cases. G*Power version 3.1.9.7. was used for sample size calculation.

Case Selection and specimen processing

This study consisted of forty formalin fixed and paraffin-embedded specimens of normal, benign and malignant SG tumors. The collected cases were selected from the archives of Oral Pathology Department, Faculty of Dentistry, Ain Shams University and General Pathology Departments, Faculties of Medicine, Ain Shams University and Cairo University.

The collected specimens consisted of control group of normal SG tissue associated with mucocele formed of 8 cases (group 1), benign group (group 2) consisted of 2 subgroups each of 8 cases including PA (group 2A) and WT (group 2B) and malignant group (group 3) consisted of 2 subgroups each of 8 cases including MEC (group 3A) and ADC (group 4A).

Sections of 5 μ m thick were cut and mounted on glass slides to confirm the diagnosis and were stained with haematoxylin and eosin stain and examined by the light microscope.

Immunohistochemical procedures

For all specimens, 4 μ m thick sections were prepared and mounted on positively charged glass slides to be immunostained. Xylene was used for deparaffinization and graded ethyl alcohol for rehydration. Sections were immersed in citrate buffer solution of pH 4.8 and before staining procedures, sections were put in the microwave oven. A universal kit (provided by Lab Vision) was used for immunohistochemical staining. Peroxidase anti- peroxidase method of immunostaining using the streptavidin-biotin system was

carried out and to block the endogenous peroxidase activity, 3% hydrogen peroxide was applied to the sections.

The immunohistochemical staining was performed using the primary antibody of monoclonal prediluted antibody against CD73 purchased from Medyasis Corporation, USA (MC0398RTU7) and polyclonal concentrated antibody against VEGFR-2 purchased from Abclonal Corporation, USA (A11127).

Sections were covered by the link antibody followed by the streptavidin labeling antibody; after rinsing with PBS, DAB chromogen was applied to the sections followed by counter stain. Then, sections were dehydrated in graded alcohol, immersed in xylene and mounted. For each positive section, four microscopic fields with the highest immunopositivity were selected and photomicrographs were captured at a magnification of 20X and 40X by digital camera (EOS 650D, Canon, Japan) which was mounted on a light microscope (BX60, Olympus, Japan). All the steps for immunohistochemical analysis were performed using image analysis software (Image J, 1.41a, NIH, USA). This study was done in vitro so the ethical committee at Faculty of Dentistry, Ain Shams University declared an exemption (FDASU-Rec EM122206).

Statistics

Data was analyzed using Statistical Package for Social Science software computer program version 26 (SPSS, Inc., Chicago, IL, USA). Quantitative data was parametric and presented as mean and standard deviation. Normal distribution of data was detected using Shapiro-Wilk test. Student's t-test (unpaired) was used for two different groups of parametric data while one way ANOVA followed by Tukey was used to compare more than two different groups of parametric data. Pearson's correlation was

used to correlate between CD73 & VEGFR-2. P value less than 0.05 was considered statistically significant.

Results

Immunohistochemical results

A- CD73:

Immunohistochemical staining with monoclonal antibody against CD73 was assessed. The positive reaction was brown in color and both membranous and/or cytoplasmic in localization. All the examined cases (100%) of normal SG, benign SG and malignant SG tumors showed positive CD73 immunostaining. The distribution of immunopositivity in normal SG control was mainly in acinar cells, myoepithelial cells, ductal epithelial cells and the endothelial cells lining the wall of some blood vessels (Fig.1A). CD73 was also positive in epithelial and myoepithelial cells of duct-like structures and masses in PA (Fig.1B). WT demonstrated positive CD73 immunoexpression in the bilayered epithelial lining and lymphoid stroma (Fig. 1C). In addition, MEC showed CD73 reaction in epidermoid cells and stromal cells (Fig.1D). The distribution of immunopositivity in ADC was clear in stromal cells and endothelial cells lining blood vessels while the expression was negative in tumor cells (Fig. 1E).

B-VEGFR-2:

Immunohistochemical staining with polyclonal antibody against VEGFR-2 was assessed. The positive reaction was brown in color membranous and/or cytoplasmic and/or nuclear in localization. VEGFR-2 immunostaining was detected in all examined cases (100%) of normal SG, benign SG and malignant SG neoplasms. Normal SG showed positivity mainly in acinar cells, myoepithelial cells, ductal epithelial cells and endothelial cells lining the wall of some blood vessels (Fig.2A). PA demonstrated immunoreaction in epithelial and

myoepithelial cells of duct-like structures and masses (Fig.2B). Whereas WT showed immunopositivity in the bilayered epithelial lining and lymphoid stroma (Fig. 2C). The distribution of immunopositivity in MEC was mainly in epidermoid cells and stromal cells (Fig.2D). ADC showed VEGFR-2 reaction in tumor cells, stromal cells and endothelial cells lining blood vessels (Fig. 2E).

Statistical Results

For CD73 and VEGFR-2 immunohistochemical expression surface area: Group (2) and Group (3) showed significant increase compared to Group (1) ($p < 0.001$, < 0.001 respectively). Whereas Group (3) showed significant increase compared to Group (2) ($p < 0.001$) (table1) & (Fig.3A). Group (2b) showed significant increase compared to Group (2a) ($p < 0.001$) (table2) & (Fig.3B). Group (3b) showed significant decrease compared to that in Group (3a) ($p < 0.001$) (table3) & (Fig.3C). CD73 showed significant positive correlation with VEGFR-2 within Group (2), Group (3) and all groups together while within Group (1) correlation was non-significant (table4) & (Fig.3D).

Table 1: Comparison of immunohistochemical expression of CD73 and VEGFR-2 between Group (1), Group (2) and Group (3).

	Groups			P value
	Group(1)	Group(2)	Group(3)	
CD73	3.66±0.29	11.34±1.31	23.79±5.66	<0.001*
Post- hoc		P1=<0.001	P2=<0.001 P3=<0.001	
VEGFR-2	5.96±1.31	20.59±5.23	44.97±5.65	<0.001*
Post-hoc		P1=<0.001	P2=<0.001 P3=<0.001	

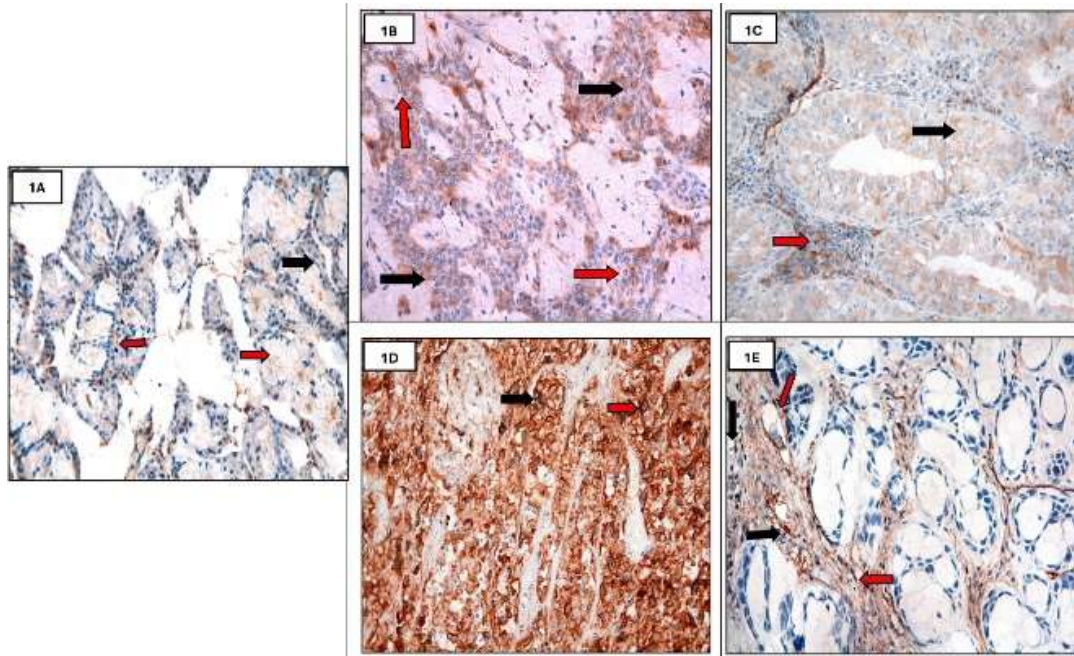


Figure 1: 1A: A photomicrograph of normal SG showing CD73 reaction in the acinar cells and myoepithelial cells (red arrows) and endothelial cells lining the walls of blood vessels (black arrow) (CD73, original magnification 40X). 1B: A photomicrograph of PA showing positive CD73 immunostaining in the cell membrane (red arrows) and cytoplasm (black arrows) of epithelial and myoepithelial cells of duct like structure and masses (CD73, original magnification 40X). 1C: A photomicrograph of WT showing positive CD73 expression in bilayered epithelial lining and lymphoid stroma, membranous (red arrow) and cytoplasmic (black arrow) (CD73, original magnification 40X). 1D: A photomicrograph of MEC showing positive CD73 immunostaining in the cell membrane (red arrow) and cytoplasm (black arrow) of epidermoid cells (CD73, original magnification 40X). 1E: A photomicrograph of ADC showing a positive CD73 reaction in the cell membrane and cytoplasm of stromal cells (black arrow) and endothelial cells lining blood vessels (red arrow) (CD73, original magnification 40X).

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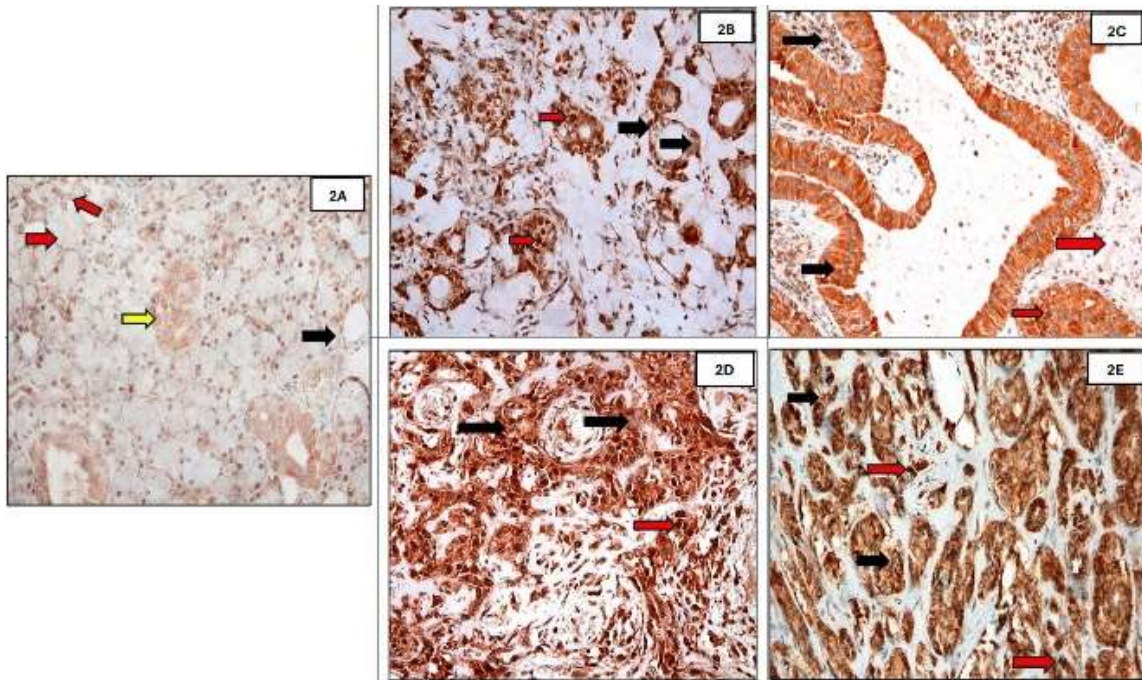


Figure 2: 2A: A photomicrograph of normal SG showing VEGFR-2 reaction in the acinar cells and myoepithelial cells (red arrows), endothelial cells lining the wall of blood vessel (black arrow) and ductal cells (yellow arrow) (VEGFR-2, original magnification 40X).

2B: A photomicrograph of PA showing positive VEGFR-2 immunostaining in the membrane and cytoplasm (black arrows) or cytoplasmic and nuclear (red arrows) of epithelial cells forming duct like structure and masses (VEGFR-2, original magnification 40X).

2C: A photomicrograph of WT showing positive VEGFR-2 immunostaining either cytoplasmic and nuclear (red arrow) or in the cell membrane and cytoplasm (black arrows) of bilayered epithelial lining and lymphoid stroma (VEGFR-2, original magnification 40X).

2D: A photomicrograph of MEC with positive VEGFR-2 expression in the cell membrane and cytoplasm (black arrows) or cytoplasmic and nuclear (red arrow) of epidermoid cells forming masses and duct-like structure (VEGFR-2, original magnification 40X).

2E: A photomicrograph of ADC showing positive VEGFR-2 immunostaining in the cell membrane and cytoplasm (black arrows) or in the cytoplasm and nucleus (red arrow) of tumor cells in solid masses (VEGFR-2, original magnification 40X).

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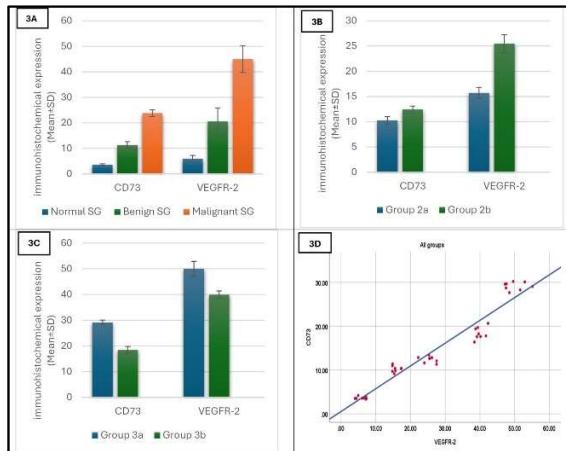


Figure 3: Figure 3A: Mean±SD of immunohistochemical expression of CD73 and VEGFR-2 between Group (1), Group(2) & Group(3). Figure 3B: Mean±SD of immunohistochemical expression of CD73 and VEGFR-2 between Group(2a) & Group(2b). Figure 3C: Mean±SD of immunohistochemical expression of CD73 and VEGFR-2 between Group(3a) & Group(3b). Figure 3D: Scatter diagram representing direct correlation between CD73 and VEGFR-2 expressions.

Table 2: Comparison of immunohistochemical expression of CD73 and VEGFR-2 between Group (2a) and Group (2b).

	Group(2a)	Group(2b)	P value
CD73	10.26±0.71	12.42±0.70	<0.001*
VEGFR-2	15.72±1.07	25.47±1.75	<0.001*

Table 3: Comparison of immunohistochemical expression of CD73 and VEGFR-2 between Group (3a) and Group (3b).

	Group(3a)	Group(3b)	P value
CD73	29.16±0.90	18.42±1.38	<0.001*
VEGFR-2	50.00±2.91	39.93±1.41	<0.001*

Table 4: Correlation coefficient value (r) and P-value between CD73 and VEGFR-2 expression in all groups.

		VEGFR-2	
		r	P
CD73	Group (1)	-0.082	0.84
	Group (2)	0.78	<0.001*
	Group (3)	0.92	<0.001*
	All Groups	0.96	<0.001*

Discussion

SGs are the organs responsible for production of saliva and are formed of acini and ducts in a complex system.¹¹ The WHO organization updated the 2022 classification of SG tumors to include 15 benign tumors and 21 malignant tumors with overlapping features and challenges for diagnosis.¹² PA is considered the most commonly occurring benign SG tumor followed by WT.¹³ Whereas MEC and ADC are the most common malignant SG neoplasms.¹⁴

CD73 is one of the immune check point inhibitory targets that play a role in adenosine production which favours growth, proliferation, invasion and angiogenesis.¹⁵ Buissere et al¹⁵ suggested that CD73 expression was obvious in many cancer types such as head and neck cancer, breast cancer, melanoma and colorectal cancer. Shah et al¹⁶ suggested that VEGFR-2 played a role in regulating the survival, proliferation, migration of endothelial cells and vascular permeability through activating different cascades.

The present study was conducted to detect the immunohistochemical expression of CD73 and VEGFR-2 in both benign and malignant salivary gland neoplasms. Also, the study aimed to detect the correlation between CD73 and VEGFR-2 if present.

Based upon the immunohistochemical results in this study, CD73 expression showed positivity in 100% of the examined cases of group (1) normal SG, group (2) benign SG and group (3) malignant SG tumors. In all three groups, the reaction was obvious in the cytoplasm and/or cell membrane. This pattern of reaction was in agreement with Ranjbar et al¹⁷ who carried out a study on benign and malignant SG neoplasms and Ren et al¹⁰ on squamous cell carcinoma.

CD73 is a dimer anchored to the plasma membrane via a glycosylphosphatidyl

inositol (GPI) which explains the membranous reaction in the cells.¹⁸ The cytoplasmic reaction is due to the presence of intracellular localization of CD73 through shedding by the action of hydrolysis by GPI anchor phospholipase or through the proteolytic cleavage by matrix metalloproteinases (MMP) leading to the release from the cell membrane.¹⁹

In the present study, CD73 expression in group (1) normal SG showed the least mean area fraction with statistically significant value (3.66 ± 0.29) when compared to group (2) and group (3). The reaction was in acinar, myoepithelial and ductal cells as well as endothelial cells lining the walls of blood vessels. This pattern of reaction was in agreement with Ranjbar et al.¹⁷ In addition, this pattern of reaction was in accordance with Virtanen et al.²⁰ who carried a study on normal breast tissue with multiple immunohistochemical markers among them was CD73, which showed expression in normal mammary glands mainly in breast epithelium, specifically in lobular and ductal epithelial cells. In normal SG, CD73 produces adenosine which is important for cell growth, survival and in case of inflammation or insult CD73 is required to cause immunosuppression and cease the inflammatory process which explains the reaction and presence of CD73 in normal SG tissue.²¹

CD73 mean area fraction in group (2) benign SG tumors was significantly higher than group (1) and less than group (3) malignant SG tumors with statistical value (11.34 ± 1.31). The mean area fraction of PA was significantly less than WT with mean area fraction (10.26 ± 0.71). PA showed CD73 expression was mainly in epithelial and myoepithelial component as well and these results were in accordance with Ranjbar et al.¹⁷

The higher expression of CD73 in PA than normal could be explained because of

the neoplastic nature of PA with high degree of epithelial cells proliferation. EGFR overexpression occurs in tumors more than normal cells since EGFR is involved in cell growth, proliferation, cell function and driver of tumorigenesis.²² Navarini et al.²³ suggested that EGFR oncogene is over expressed in epithelial and myoepithelial cells of PA as it is considered a benign tumor and involved in proliferation. When EGFR gene is activated, a sequence of pathways occurs resulting in activation of CD73 and vice versa.²⁴ Among these pathways are the MAPK and PI3K/AKT signaling pathways, all of which led to cell growth and survival.²⁴ This relation between CD73 and EGFR could explain the expression of CD73 in PA since both affect each other's expression.

The current study was the first to assess the immunohistochemical expression of CD73 in WT. The present study results showed a statistically significant value higher than PA and less than malignant tumors (12.42 ± 0.70). The expression was in the lymphoid stroma more than in the epithelial layer. The pattern of distribution may be due to the fact that CD73 is a lymphocyte differentiation antigen and is expressed widely on many immune cells such as B and T cells.⁶

Another study conducted by Qotrunnada et al.²⁵ in which the effect of cytotoxic T lymphocyte antigen 4 (CTLA-4) (which is one of the inhibitory targets), on WT growth was discussed. They found that the expression of CTLA-4 was more in the lymphoid stroma than the epithelium with a high expression in B and T cells, which is in accordance with the results of the present study. This could explain the pattern of CD73 in WT in the current study. In addition, Aoki and Tanaka²⁶ suggested that the presence of lymphocytes in WT stroma indicated chronic inflammation therefore enhancing CD73 production.

CD73 expression in group (3) malignant tumors showed the highest statistically significant value (23.79 ± 5.66) compared to the other groups, this was in accordance with Ranjbar et al¹⁷. These results were explained by Kang et al²⁷ who suggested that malignant tumors were more aggressive with more hypoxia and induction of hypoxia inducible factor (HIF). The produced HIF promoted the production of CD73 to promote cellular adaptation for hypoxic microenvironment.¹⁵ Ghalamfarsa²⁸ also suggested that CD73 overexpression was associated with invasion, lymph node metastases and poor survival in different types of neoplasms which explained the higher expression in malignant SG neoplasms.

The present study showed CD73 expression in MEC in the epidermoid cells forming masses, sheets and duct-like structures and in stromal cells, with mean area fraction that was statistically higher than ADC (29.16 ± 0.90). Ranjbar et al¹⁷ agreed with the current study results, in which MEC showed expression of CD73 in the epidermoid cells and stromal cells and their results were higher in MEC than ADC. The difference in mean area fraction, although both are malignant lesions, could be explained in the present study as most of the ADC cases were of low grade nature because they were mainly cribriform while most of MEC cases were of high grade nature because case were mainly solid. In accordance with the current study results, Ren et al¹⁰ showed that CD73 expression was higher in poorly differentiated squamous cell carcinoma than well differentiated squamous cell carcinoma.

In the current study, the expression of CD73 in ADC was positive in stromal cells and endothelial cells lining blood vessels and negative in tumor cells and mean area fraction (18.42 ± 1.38). This pattern of reaction was seen in a study by Bauer et al²⁹ who showed that the expression of CD73 in

MEC was mainly in immune cells (B and T cells) rather than tumor cells which was in accordance with the result of the present study. A study conducted by Jiang et al³⁰ in which the CD73 expression in non-small cell lung cancer was discussed, found that the expression of CD73 was different in the reaction pattern with heterogeneity in expression between one cell and another also between an area and the neighboring. These results were explained as CD73 showed mutations and deletion in some cells. They also suggested that this occurred due to clonal evolution which is a process by which different subclones from the cell of origin within the tumor change overtime.³⁰

In accordance with the results of CD73 immuno-expression, VEGFR-2 immunoexpression was also positive in 100% of the examined cases of group (1), group (2) and group (3). In all three groups, the reaction was mainly in the cytoplasm and/or cell membrane. The pattern of reaction was in agreement with the conducted studies by Faur et al³¹ on SG tumors, Edirisingh et al³² on squamous cell carcinoma and Mei et al³³ on renal cell carcinoma. In addition, the current study showed nuclear reactions where this reaction was also seen in squamous cell carcinoma in lung in a study done by Holzer et al³⁴. VEGFR-2 is a membrane-associated tyrosine kinase receptor, which explains the membranous reaction. Whereas the pattern of nuclear and cytoplasmic reaction occurred because VEGFR-2 can dynamically internalize and translocate to the cytoplasmic and nuclear compartments for further activation.³⁴ VEGFR-2 requires an internal ligand for activation that is not accessible in the extracellular compartment.³⁴

The normal SG showed the least VEGFR-2 reaction compared to group (2) and group (3) with statistically significant value of mean area fraction (5.96 ± 1.31). The reaction was in acinar cells, myoepithelial cells, ductal cells and endothelial cells lining

walls of blood vessels. The present study results were in line with Faur et al³¹ and Błochowiak et al⁹ who suggested that VEGFR-2 existed normally to maintain vascularity and normal tissue hemostasis, and that its levels might change in response to pathological conditions as tumors.

VEGFR-2 mean area fraction in group (2) benign SG tumors was significantly higher than group (1) and less than group (3) malignant SG tumors with statistical value (20.59 ± 5.23) and this was in accordance with Faur et al³¹. The expression of VEGFR-2 in PA was in epithelial and myoepithelial components with a statistical value of mean area fraction (15.72 ± 1.07) when compared to WT. Pleomorphic adenoma gene 1 (PLAG1) is an oncogene in pleomorphic adenoma which is involved in cell proliferation and tumor growth through the activation of PI3k/AKT pathway which is also involved in the regulation of VEGFR-2 expression. Therefore, PLAG1 gene in PA indirectly activates that angiogenic process mediated by VEGFR-2, a fact which was in accordance with the results of the present study.^{35, 36}

WT showed reaction in the epithelial component more than lymphoid stroma with a higher mean area fraction than PA in VEGFR-2 with a significant statistical value (25.47 ± 1.75). Aoki and Tanaka²⁶ suggested that WT was characterized by the presence of oncocytic epithelial cells that were aging cells with more status of chronic inflammation which promoted more angiogenesis and lymphoid stroma with lymphocytes and other immune cells that secreted cytokines and growth factors further stimulating angiogenesis. This could explain the findings of the present study. The higher expression of VEGFR-2 in WT than PA could be attributed to that WT was a tumor with more vascular density than PA, also WT is thought to arise from residual SG tissue within lymph nodes with more vascularized

structure due to pre-existing vascular networks.³⁷

VEGFR-2 expression in group (3) malignant tumors was higher compared to group (1) and (2) with a statistically significant value (44.97 ± 5.65). This was in agreement with the study conducted by Faur et al³¹ on SG neoplasms that suggested that the expression of VEGFR-2 was higher in malignant SG tumors compared to benign tumors. MEC showed VEGFR-2 expression in epidermoid cells and stromal cells with a statistical value of mean area fraction (50.00 ± 2.91) when compared to ADC. Costa et al³⁸ suggested that VEGFR-2 expression was affected by molecular alterations where MEC often had MAML2 gene mutation which played a role in upregulating the activity of VEGF, which was also in line with the result of the current study and supporting the higher value of mean area fraction of VEGFR-2 expression in MEC than ADC.

The immunohistochemical expression of VEGFR-2 in ADC was mainly in tumor cells in addition to stromal cells and endothelial cells lining blood vessels. This pattern of reaction was in accordance with Faur et al³¹. ADC showed mean area fraction value (39.93 ± 1.41) which was less than MEC, as mentioned before this could be explained because of the type of cases in the present study, where ADC cases were mostly of low-grade nature with more cribriform pattern. Xu et al³⁹ showed that VEGFR-2 expression was higher in poorly differentiated squamous cell carcinoma than well differentiated squamous cell carcinoma. Mei et al³³ suggested that this overexpression in malignant neoplasms was related to progression, invasion, metastasis and poor survival. In agreement with the current study, VEGFR-2 overexpression is also observed in different types of malignant tumors such as breast cancer, squamous cell carcinoma, malignant melanoma, B-cell lymphoma.³²

The present study was the first to discuss the relation between benign and malignant SG neoplasms through the assessment of immunohistochemical expression of CD73 and VEGFR-2. There was a statistically significant direct correlation between CD73 and VEGFR-2 immunoexpression in benign SG and malignant SG tumors. This was in accordance with a study carried out on renal cell carcinoma by Mei et al³³. Koszałka et al⁴⁰ suggested that adenosine produced by CD73 played a role in angiogenesis through regulation of proangiogenic factors through binding to A₂AR receptors subtypes (A_{2A}AR and A_{2B}AR) which in turn activated many angiogenic factors as VEGFR-2. This explained the direct correlation between CD73 and VEGFR-2 proved in the current study.

Conclusion

Based upon the immunohistochemical results of the current study, the high expression of CD73 and VEGFR-2 indicates poor prognosis and might have a promising role as prognostic markers for SG neoplasms. There is a direct correlation between both markers.

Declarations

Funding: No funding from any funding agency totally funded by the authors.

Data availability: The datasets used and analyzed during the present study are available from the corresponding author upon reasonable request.

Ethical approval and consent to participate: This study was done in vitro so the ethical committee at Faculty of Dentistry, Ain Shams University declared an exemption (FDASU-Rec EM122206).

Competing interests: There is no conflict of interest declared by the authors.

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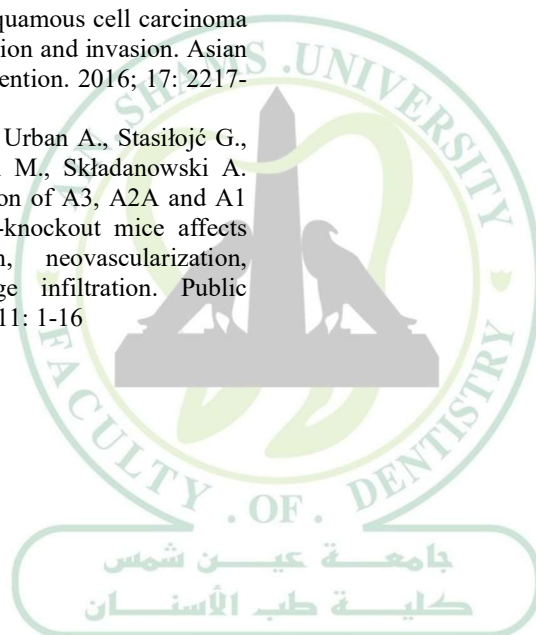
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