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Original article

The role of fibrinogen-like protein 1 in immune escape and tumor growth mechanism of Warthin's tumor

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ABSTRACT

Background: *One of the benign tumors of the salivary glands is Warthin's Tumor (WT), which consists of cystic bilayer papillary epithelial cells accompanied by the presence of a lymphoid stroma. Several cases have been reported to turn malignant. One of the markers developed to identify tumor proteins is fibrinogen-like protein 1 (FGL1). Along with lymphocyte-activation gene 3 (LAG-3), FGL1 establishes an immune checkpoint pathway that plays a role in the mechanism of tumor immune escape. Not much has been reported regarding FGL1 expression in WT, but some studies have reported that its expression is associated with tumor growth.* ***Purpose:*** *This study aims to analyze the location of FGL1 expression in WT and its relation to the mechanism of tumor immune release through the interaction between FGL1 and LAG-3.* ***Methods:*** *Cases of WT (n = 11) and breast cancer (n = 1) were used as positive controls. All cases were stained with hematoxylin-eosin and Recombinant Anti-FGL1 antibody. The FGL1 expression was observed in the cell membrane, cytoplasm, and lymphoid stroma. The results are presented in the form of figures.* ***Results:*** *All cases of WT expressed FGL1 in the cell membrane, cytoplasm, and lymphoid stroma. Its expression in the cell membrane and cytoplasm is possibly related to the process of tumorigenesis and the increasing size of the lesion. Additionally, its expression is seen in the lymphoid stroma, which is closely related to immune escape by inhibiting lymphocytes against tumor cells.* ***Conclusion:*** *Warthin's tumor cells express FGL1, and this expression plays a role in tumor immune escape mechanisms and tumor growth.*

Keywords: *fibrinogen-like protein 1; immune escape; Warthin's tumor*
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INTRODUCTION

Warthin's Tumor (WT) is the second most frequently identified benign salivary gland tumor.[1,2] These tumors often present with large lesion diameters, high pain intensity, high recurrence rate, and often occur in old age.[1] Additionally, WT can transform into malignancy, and the pathogenesis of this transformation remains unknown.[3–5] Several studies have shown the transformation of WT into malignant tumors, such as squamous cell carcinoma, mucoepidermoid carcinoma, adeno squamous carcinoma, and Merkel cell carcinoma.[6–8] This malignancy presents negative manifestations, including speaking and swallowing dysfunctions, pain, facial deformities, and even death.[9,10] Hence, biomarkers are necessary to determine changes in WT toward malignancy by examining the immune checkpoint pathway.[11,12] However, some highly expressed proteins of the immune checkpoint, such as lymphocyte-activation gene 3 (LAG-3), inhibit immune cells and indirectly support tumor growth.[13,14] Previous research conducted by Qotrunnada et al.[15] showed the expression of cytotoxic T-lymphocyte antigen 4 in the solid components and germinal center lymphoid stroma of WT, which is related to T cell activity inhibition against tumor cells.

Fibrinogen-like protein 1 (FGL1) was recently discovered as the main LAG-3 ligand forming the immune checkpoint pathway.[16–18] This pathway is crucial in the mechanism of immune escape or the ability of tumor cells to evade immune cells.[16,17] The interaction between FGL1 and

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LAG-3 inhibits activation and weakens T-cell cytotoxicity, making it difficult for tumor cells to be destroyed.[19]

Under normal conditions, FGL1 is physiologically secreted by hepatocyte cells.[19,20] However, FGL1 was also highly expressed in various tumors such as lung, prostate, melanoma, colorectal, breast, and brain tumors.[16,19] This high expression of FGL1 in tumor cells contributes to the growth and development of neoplasms causes host lymphocyte cells (Tumor Infiltrating lymphocytes) to become cell exhausted, a condition in which lymphocytes lose their ability to kill tumor cells. Therefore, monoclonal antibody inhibitor FGL1 (anti-FGL1) immunotherapy was developed by blocking the interaction of FGL1 with LAG-3, stimulating T cell activation and restoring the antitumor response.[18]

Based on the above description, the aim of this research is to analyze FGL1 expression in WT, determine its location of expression, and clarify its pathogenesis related to the immune escape mechanism and tumor growth. In addition, FGL1 expression and its relationship with immune escape have not been widely reported or published in tumors of the salivary gland, especially in WT.

MATERIALS AND METHODS

This research is an analytical observational study using a cross-sectional design and has obtained approval from the ethics committee with No. 1654/UN25.8/KEPK/DL/2022 from the Dentistry Faculty of Universitas Jember. The research sample comprised paraffin blocks of WT patients from 2015 to 2022 at the Laboratory of Anatomical Pathology of Dr. Soebandi General Hospital, Jember, East Java. A purposive total sampling technique was employed in the sample selection, obtaining 11 cases of WT and 1 case of breast cancer, which was used as a positive control for antibodies. All paraffin-embedded tissue blocks were cut to a thickness of 4 µm using a sliding microtome for hematoxylin-eosin staining and immunohistochemistry (IHC) staining.

Standard procedures were followed in IHC staining. Tissue slides were deparaffinized, rehydrated, and washed with phosphate-buffered saline (PBS). They were then immersed in citrate buffer (pH 6) for antigen retrieval and autoclaved for 15 minutes at 121°C, followed by cooling at room temperature for 30 minutes. Subsequently, all slides were rinsed with PBS. All sample slides were prevented from nonspecific binding between antibodies and tissue by dripping hydrogen peroxide onto the tissue slide and incubating it for 30 minutes at room temperature, followed by rinsing with PBS. Next, the protein block was incubated for 24 hours at 4°C to block nonspecific background staining and rinsed with PBS afterward. The tissue slide surfaces were covered with primary antibody Recombinant FGL1 antibody (EPR24018-27) (ab275093) (Abcam Inc., Cambridge, UK) at a ratio of 1:500 and then incubated for 24 hours at 4˚C and rinsed with PBS. Biotinylated Goat Anti-Rabbit IgG (ab64256) (Abcam Inc., Cambridge, UK) was applied as secondary antibodies to the tissue slides for 10 minutes at room temperature, which was followed by rinsing with PBS. Streptavidin peroxidase was then added for 10 minutes at room temperature. A drop of 3,3' diaminobenzidine (DAB) substrate kit (ab64238) (Abcam Inc., Cambridge, UK) solution was added to cover the surface of the tissue section and incubated for 10 minutes at room temperature. Counterstaining of sample slides was performed using Mayer's Hematoxylin (MHS32) (Sigma-Aldrich, Merck, Darmstadt, Germany) for one minute, and then cleaning and mounting took place. This research utilized qualitative data. The immunopositive expression of FGL1 was determined by the appearance of immunopositivity on cell membranes, cytoplasm, and lymphoid stroma. Two observers conducted the observation using a binocular microscope at 100x, 400x, and 1000x magnification. The images were digitally scanned using Optilab (Miconos®, Yogyakarta, Indonesia).

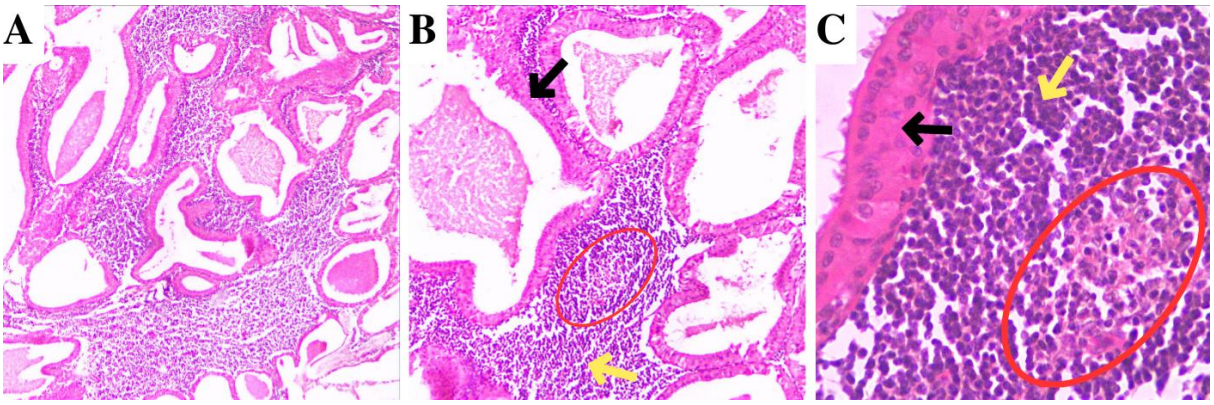

Figure 1. Warthin's tumor (WT) cells on hematoxylin-eosin staining. Pictures A-C are the histological appearance of WT cells on hematoxylin-eosin staining with magnifications of 40x, 100x, and 400x, respectively. The histopathological images show the structure of WT, characterized by finger-shaped bilayer epithelial cells surrounding the cystic space, or the so-called papillary cystic (black arrow), and lymphoid stroma consisting of a solid lymphoid component (yellow arrow) and germinal center (red circle).

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RESULTS

Initial histopathological observations using HE staining revealed that all samples (100%) were WT and characterized by the presence of papillary cystic epithelial cells arranged in a bilayer. These cells consisted of columnar cells in the inner layer surrounded by basaloid cells, along with lymphoid tissue containing germinal centers in the tumor stroma (Figure 1). Based on the results of IHC staining using anti-FGL1, the positive control expressed FGL1, which was indicated by the presence of a brown color in the cytoplasm of grade III breast cancer cells (Figure 2).

All samples of WT tissues using IHC staining with the FGL1 antibody were immunopositive for FGL1 on epithelial cells (100%), both on the membrane and in the cytoplasm (Figure 3). In the lymphoid stroma, 7 out of 11 samples (63%) exhibited immunopositivity in the solid lymphoid component, while 10 out of 11 samples (91%) showed immunopositivity in the germinal center (Figure 4). Thus, it is evident that WT is immunopositive for FGL1 expression (Table 1).

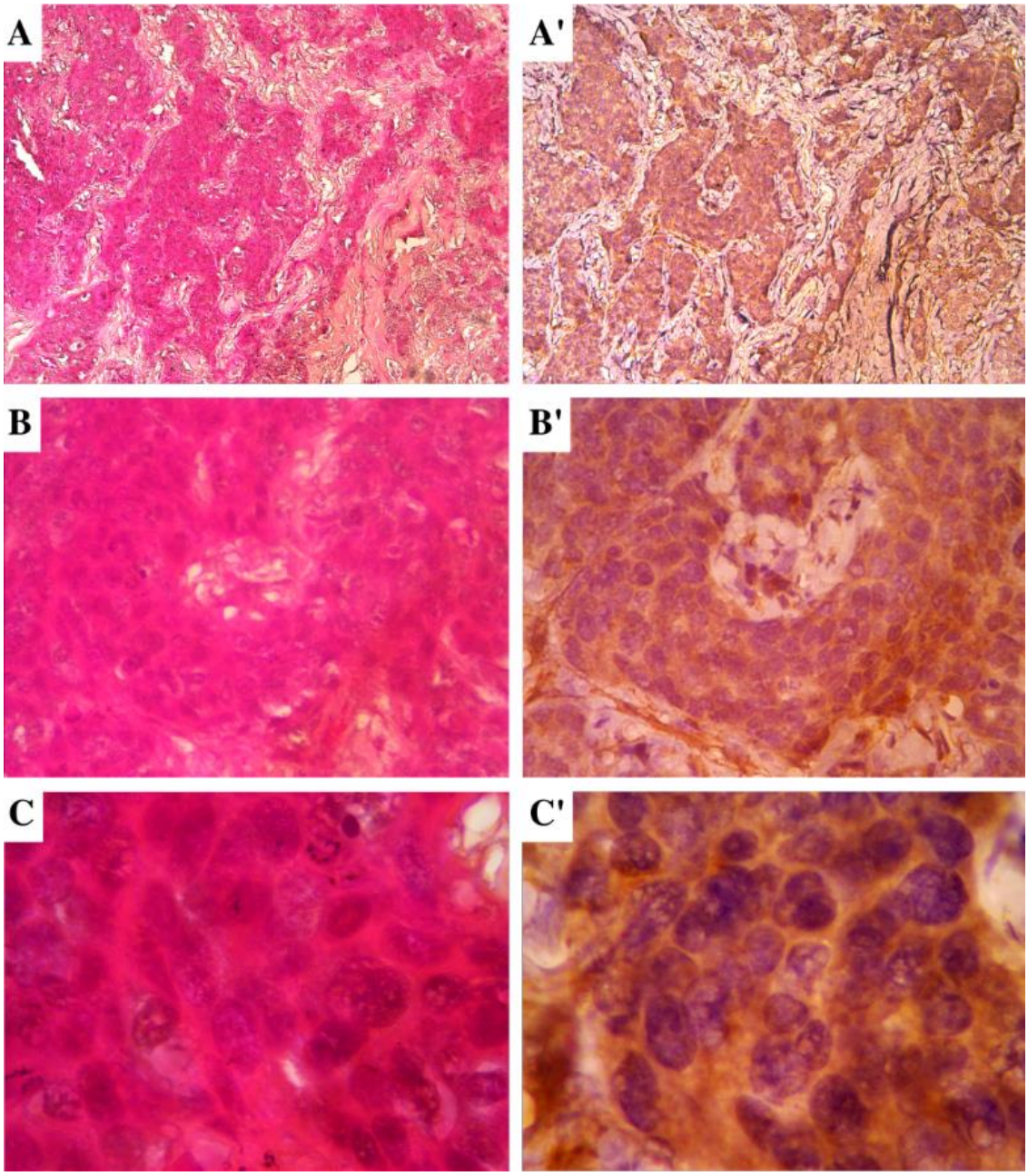

Figure 2. Pictures A-C depict fibrinogen-like protein 1 (FGL1) expression in the cytoplasm of grade III breast cancer, serving as the positive control, on hematoxylin-eosin staining with magnifications of 100x, 400x, and 1000x, respectively. Pictures A'-C' show FGL1 immunopositive expression in the cytoplasm of tumor cells at magnifications of 100x, 400x, and 1000x, respectively.

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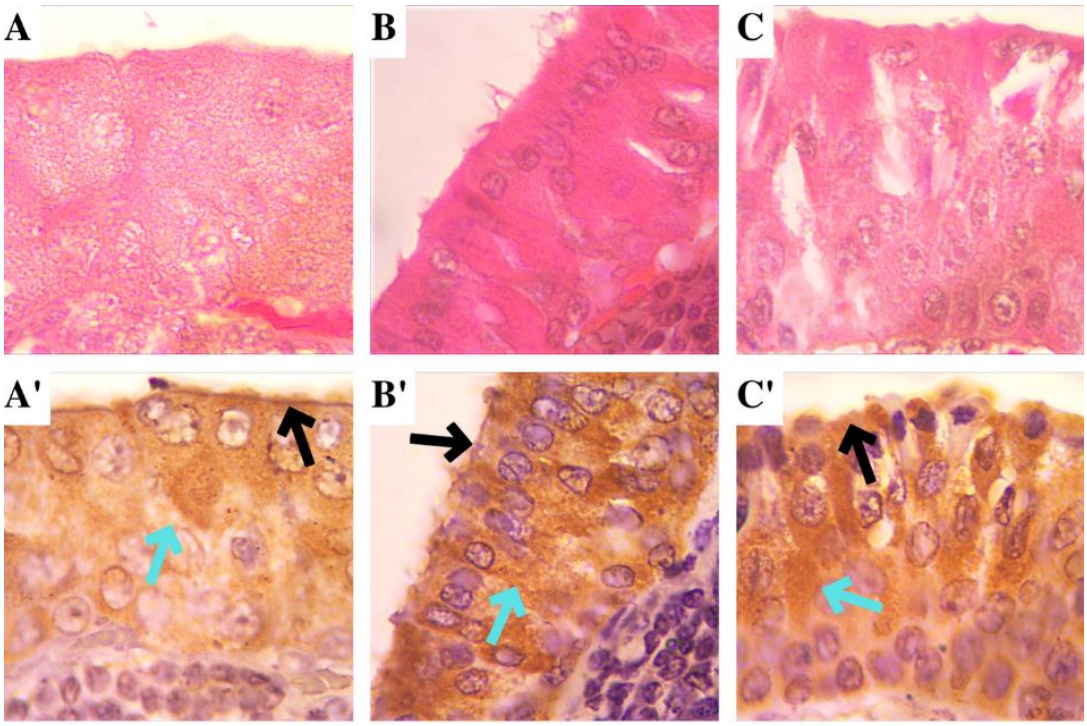

Figure 3. Pictures A-C show fibrinogen-like protein 1 (FGL1) expression in epithelial cells from three cases of Warthin's tumor on hematoxylin-eosin staining at 1000x magnification. Pictures A'-C' show FGL1 in Warthin tumor epithelial cells at 1000x magnification. FGL1 expression appears immunopositive, both in the epithelial cell membrane (black arrow) and the cytoplasm of epithelial cells (blue arrow).

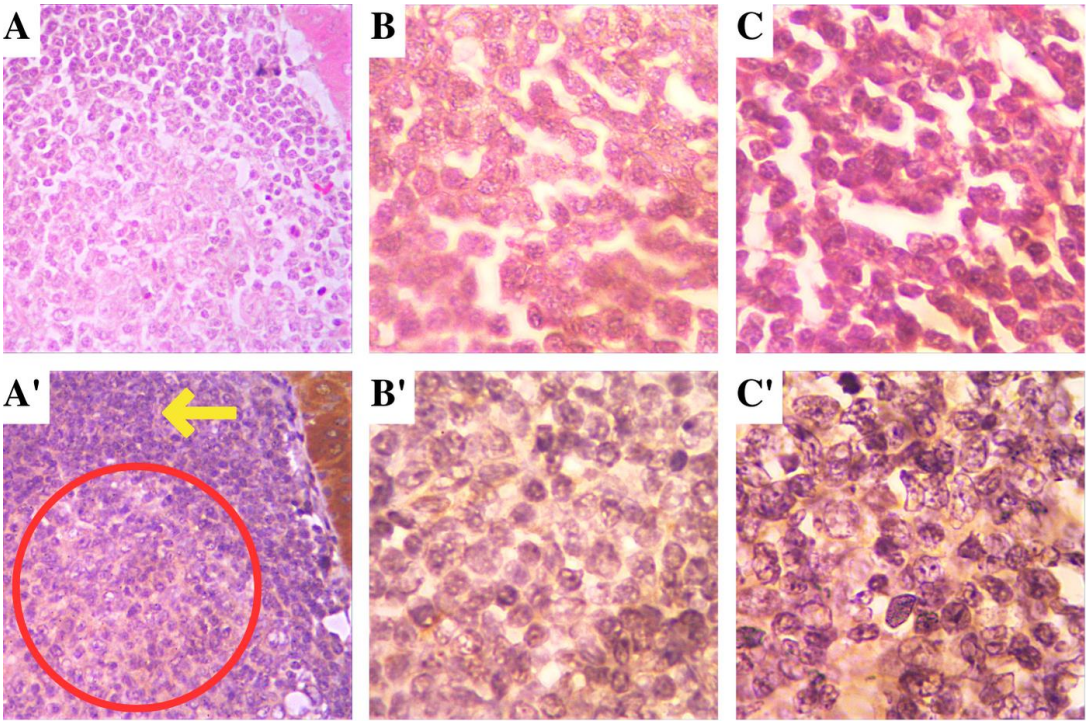

Figure 4. Fibrinogen-like protein 1 (FGL1) expression in Warthin's tumor (WT) lymphoid stroma (Pictures A-C). The appearance of Warthin's tumor on hematoxylin-eosin staining, at 400x, 100x, and 1000x magnification. Picture A' shows FGL1 expression in the lymphoid stroma, which is composed of a solid lymphoid component and a germinal center at 400x magnification. Picture B' shows FGL1 expression in the solid lymphoid component at 1000x magnification. Picture C' depicts FGL1 expression in the germinal center at 1000x magnification. In the immunohistochemistry staining, FGL1 expression appears immunopositive. FGL1 expression is seen in the solid lymphoid component (yellow arrow) and the germinal center (red circle).

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Table 1. Results of anti-FGL1 immunohistochemistry staining analysis in Warthin's tumors

Sample numbers	Histopathologic Diagnosis	FGL1 Expression			
		Tumor Epithelium		Lymphoid Stroma	
		Membrane	Cytoplasm	Solid Lymphoid Component	Germinal Center
1	Warthin's tumor	+	+	+	+
2	Warthin's tumor	+	+	+	+
3	Warthin's tumor	+	+	-	+
4	Warthin's tumor	+	+	-	+
5	Warthin's tumor	+	+	+	+
6	Warthin's tumor	+	+	+	+
7	Warthin's tumor	+	+	+	+
8	Warthin's tumor	+	+	-	+
9	Warthin's tumor	+	+	-	+
10	Warthin's tumor	+	+	+	+
11	Warthin's tumor	+	+	+	+

DISCUSSION

The results of IHC anti-FGL1 staining on 11 samples identified as WT tissue showed FGL1 expression in all tumor epithelial and stromal cells. This staining, with a positive control for comparison, confirms that WT expresses FGL1. Normally, FGL1 is secreted by hepatocyte cells of the liver.[19,20] However, it is possible that WT cells also produce FGL1.[16] According to Qian et al.,[16] the gene that produces FGL1 is located on human chromosome 8p22 and is present in all human somatic cells, although normally only hepatocyte cells express it. The FGL1 gene functions as a protective factor to promote growth as cells regenerate. However, the IHC staining revealed FGL1 expression in the membrane and cytoplasm of WT cells, suggesting that these cells may produce FGL1 in a soluble form and can float freely in the cytoplasm or be a component of cell membranes. This is in accordance with the research of Wang et al.,[18] which state that FGL1 was detected in dissolved form.

The strong expression of FGL1 in WT epithelial cells is associated with an important role of FGL1 in tumorogenesis.[16] According to Qian et al.,[16] in some tumors, FGL1 is involved in regulating proliferation, apoptosis resistance, sensitivity to radiation, supporting epithelial-mesenchymal transition (EMT) processes, neovascularization, metastasis, immune regulation, and drug resistance, all of which are closely associated with tumor growth. Tumor cells may express high amounts of FGL1 because FGL1 is necessary for proliferation, facilitating the expansion and enlargement of the lesion. This is consistent with the study of Chan et al.,[1] which state that the diameter of the WT lesion is larger than other salivary gland tumors. In addition, FGL1 affects apoptosis resistance, causing WT cells to continue dividing uncontrollably and making them difficult to stop. FGL1 also supports the process of EMT, neovascularization, and metastasis, which may be the cause of WT cells transforming into malignancy, as observed in several cases.[16] The many important roles of FGL1 are thought to cause a high expression of FGL1 in WT epithelial cells. However, further research needs to be done to determine the exact mechanisms involved.

The presence of FGL1 expression in the lymphoid tissue of WT stroma may be related to the mechanism of immune escape or the ability of tumor cells to evade immune cells. According to Kuzenko et al.,[3] the lymphoid stroma is composed of B lymphocytes, T lymphocytes, and natural killer cells. FGL1 expression in the lymphoid stroma is thought to play a role in the immune escape mechanism because FGL1 is the main ligand of LAG-3.[16–18] Normally, LAG-3 inhibits immune cells' work so that autoimmunity does not occur.[14,21] However, the higher the expression of FGL1, the higher the probability that the amount of LAG-3 can bind. The high LAG-3, activated by binding to FGL1, causes the lymphocytes it hosts to experience cell exhaustion, a condition in which lymphocytes lose their ability to kill tumor cells.[22] Tumor cells that are not killed will continue to proliferate. Thus, FGL1 expressed in the lymphoid stroma of WT may play a role in the mechanism of pathogenesis of immune escape. However, further research is needed to confirm the presence of LAG-3 expression in the lymphoid stroma of WT using the double staining immunohistochemistry method, namely IHC staining using two different sets of antibodies.

Moreover, four out of eleven samples did not express FGL1 in the solid lymphoid component of WT. Several possibilities can cause the non-expression of FGL1. The first possibility is associated with the constituent components, namely T lymphocyte cells. According to Ruffo et al.,[23] LAG-3 in resting T cells (resting T cells) is degraded and stored in the lysosomal compartment. This degradation process is a step to limit the LAG-3 expression on the surface of T cells. The lack of expression of FGL1 could be that T cells are dormant or inactive, which prevents FGL1 from binding to LAG-3. The second possibility is related to the suspected stop codon mutation in WT cells so that they cannot produce FGL1. This possibility is likely the cause of FGL1 not being expressed in the four WT samples. Further research is necessary, as this study was unable to explain the gene mutations associated with FGL1 overexpression and the inability of tumor-infiltrating lymphocytes to transform WT into malignancy.

In conclusion, the expression of FGL1 in the cytoplasm and epithelial cells membrane may be necessary for

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proliferation and apoptosis inhibition, while FGL1 expression in the lymphoid stroma, both in the solid lymphoid component and the germinal center of WT, likely plays important roles in immune escape by inhibiting lymphocyte activity against tumor cells. This mechanism occurs due to the overexpression of FGL1 binding to LAG-3.

REFERENCES

1. Chan W-H, Lee K-W, Chiang F-Y, Ho K-Y, Chai C-Y, Kuo W-R. Features of parotid gland diseases and surgical results in southern Taiwan. Kaohsiung J Med Sci. 2010; 26(9): 483–92.
2. Song K, Cotelingam JD, Lowery-Nordberg M, Sun W. Characterization of the lymphoid stroma in Warthin's tumor of salivary gland by immunohistochemistry, heavy chain gene and Bcl-2 gene rearrangement. Am J Transl Res. 2009; 1(4): 352–7.
3. Kuzenko Y V., Romanuk AM, Dyachenko OO, Hudymenko O. Pathogenesis of Warthin's tumors. Interv Med Appl Sci. 2016; 8(2): 41–8.
4. Rachmad N, Gofur P, Rachmadani A, Gofur P, Nur R, Putra R. Favorable treatment for rare case tumor in oral cavity: A whartin's tumor review article. Int J Dent Sci. 2021; 3(1): 26–31.
5. Raghu AR, Rehani S, Bishen KA, Sagari S. Warthin's tumour: A case report and review on pathogenesis and its histological subtypes. J Clin Diagn Res. 2014; 8(9): ZD37-40.
6. Shou L, Chen X, Yang J, Jiang Y. Malignant transformation of Warthin's tumor into squamous cell carcinoma: A case report. Oncol Lett. 2022; 24(1): 201.
7. Chulam TC, Noronha Francisco AL, Goncalves Filho J, Pinto Alves CA, Kowalski LP. Warthin's tumour of the parotid gland: our experience. Acta Otorhinolaryngol Ital. 2013; 33(6): 393–7.
8. Nguyen KA, Thai TA, Giang CT. Malignant transformation in a parotid Warthin's tumor: Clinical features and histopathological examination. J Cancer Biol Res. 2018; 6(1): 1115.
9. Shirai K, Kawashima M, Saitoh J-I, Abe T, Fukata K, Shigeta Y, Irie D, Shiba S, Okano N, Ohno T, Nakano T. Clinical outcomes using carbon-ion radiotherapy and dose-volume histogram comparison between carbon-ion radiotherapy and photon therapy for T2b-4N0M0 non-small cell lung cancer-A pilot study. PLoS One. 2017; 12(4): e0175589.
10. Valdez JA, Brennan MT. Impact of oral cancer on quality of life. Dent Clin North Am. 2018; 62(1): 143–54.
11. Li N, Hou X, Huang S, Tai R, Lei L, Li S, Abuliz A, Wang G, Yang S. Biomarkers related to immune checkpoint inhibitors therapy. Biomed Pharmacother. 2022; 147: 112470.
12. Bai R, Lv Z, Xu D, Cui J. Predictive biomarkers for cancer immunotherapy with immune checkpoint inhibitors. Biomark Res. 2020; 8: 34.
13. Huo J-L, Wang Y-T, Fu W-J, Lu N, Liu Z-S. The promising immune checkpoint LAG-3 in cancer immunotherapy: from basic research to clinical application. Front Immunol. 2022; 13: 956090.
14. Maruhashi T, Sugiura D, Okazaki IM, Okazaki T. LAG-3: from molecular functions to clinical applications. J Immunother cancer. 2020; 8(2).
15. Qotrunnada AN, Indriana T, Kosasih J, Margaretha M, Syafriadi M. Role of cytotoxic T-lymphocyte antigen 4 (CTLA-4) expression in the pathogenesis of Warthin's tumor growth. Dent J. 2022; 55(4): 194–9.
16. Qian W, Zhao M, Wang R, Li H. Fibrinogen-like protein 1 (FGL1): the next immune checkpoint target. J Hematol Oncol. 2021; 14(1): 1–17.
17. Shi A-P, Tang X-Y, Xiong Y-L, Zheng K-F, Liu Y-J, Shi X-G, Lv Y, Jiang T, Ma N, Zhao J-B. Immune checkpoint LAG3 and its ligand FGL1 in cancer. Front Immunol. 2022; 12: 785091.
18. Wang J, Sanmamed MF, Datar I, Su TT, Ji L, Sun J, Chen L, Chen Y, Zhu G, Yin W, Zheng L, Zhou T, Badri T, Yao S, Zhu S, Boto A, Sznol M, Melero I, Vignali DAA, Schalper K, Chen L. Fibrinogen-like protein 1 is a major immune inhibitory ligand of LAG-3. Cell. 2019; 176(1–2): 334-347.e12.
19. Yu J, Li J, Shen J, Du F, Wu X, Li M, Chen Y, Cho CH, Li X, Xiao Z, Zhao Y. The role of fibrinogen-like proteins in cancer. Int J Biol Sci. 2021; 17(4): 1079–87.
20. Lin W-W, Ho K-W, Su H-H, Fang T-F, Tzou S-C, Chen I-J, Lu Y-C, Chang M-S, Tsai Y-C, Liu E-S, Su Y-C, Wang Y-T, Cheng T-L, Huang H-K. Fibrinogen-like protein 1 serves as an anti-inflammatory agent for collagen-induced arthritis therapy in mice. Front Immunol. 2021; 12: 767868.
21. Graydon CG, Mohideen S, Fowke KR. LAG3's enigmatic mechanism of action. Front Immunol. 2021; 11: 615317.
22. Watowich MB, Gilbert MR, Larion M. T cell exhaustion in malignant gliomas. Trends in cancer. 2023; 9(4): 270–92.
23. Ruffo E, Wu RC, Bruno TC, Workman CJ, Vignali DAA. Lymphocyte-activation gene 3 (LAG3): The next immune checkpoint receptor. Semin Immunol. 2019; 42: 101305.

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