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Original Article

Tumor-infiltrating CD20+ B lymphocyte: evaluation and association with clinical and pathological characteristics in oral squamous cell carcinoma

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Abstract

B lymphocytes have garnered significant attention in recent research about their function in tumor immunity. Tumor Infiltrating B cells (TIBs) refer to B lymphocytes that enter the tissues of tumors. Different cellular components interplay in the environment of tumor and modulate the response of antitumor immune dynamically. A Dual role for TIBs is detected in tumor immunity regulation instead of just tumor promotion or inhibition .The current study aimed to assess tumor-infiltrating B lymphocytes and their correlation with clinical outcomes. Immunohistochemical analysis of B Lymphocytes in stromal/intratumoral regions was performed in 40 OSCC specimens by using CD20 antibodies. Expression of the marker and their relationship with clinicopathological parameters was evaluated by using of Independent t-test and Analysis of Variance (ANOVA) (two-tailed). Data analysis demonstrates a significant association of stromal and intratumoral CD20+ B lymphocyte infiltration with well-differentiated lesions ($P < 0.05$) and stage I cases ($P>0.05$). In addition to a significant correlation of stromal infiltration of CD20+B lymphocytes with lymph node metastasis ($P=0.01$). The results suggest that CD20+ B lymphocytes play an important role in OSCC, where higher infiltration of CD20+ B cells in stromal regions, particularly in cases with lymph node involvement, may be used as a prognostic indicator and may aid in determining whether the use of B lymphocyte as therapeutic targets in OSCC.

Keywords: CD20+, B lymphocyte, Tumor-infiltrating B lymphocytes, OSCC, Oral cancer, Oral squamous cell carcinoma.

1. Introduction

Oral cancer is a malignant neoplasm that occurs in the oral cavity and lips. Oral squamous cell carcinoma constitutes the predominant health issue in developing nations, leading to death, and constitutes about 95% of all oral cavity carcinomas with squamous cell type [1, 2]. Oral Squamous cell carcinoma is defined traditionally by this name due to its position in the oral region, where its form approximately 90% of all cancers are derived histologically from squamous cells [3]. Oral squamous cell carcinoma (OSCC) is characterized by a high death rate, aggressive growth style, localized invasion, and spread to cervical lymph nodes [4].

The rate of metastasis of OSCC to lymph nodes is high at early stages so it's considered an aggressive cancer [5]. The immunological system has a crucial function in the prevention of cancers. There exist intricate immunological responses from both the host and the tumor. The principal function of an immune system is to eradicate invading cells. Consequently, comprehending the immune response inside the tumor the microenvironment was highly significant [6]. B cells that can make up as much as 25% to 40% of all cells are distinct carcinoma forms [7, 8].

B lymphocytes originate from hematopoietic stem cells and are consequently produced in the bone marrow [9]. About 10-20% of the circulating peripheral lymphocyte population comprises mature B cells, which produce antibodies in the humoral immune system. Additionally, B cells secrete cytokine, serve as antigen-presenting cells (APCs), and disseminate regulating substances. B lymphocytes recognize antigens through the B lymphocyte receptors complexes embedded within the cellular membranes. [10]. Despite their smaller population compared to T lymphocytes, B lymphocytes form a part of the tumor-infiltrating lymphocyte component [11].

Data indicate that B lymphocytes may significantly contribute to anti-tumor immunity in conjunction with different immune cells, such as macrophages and T lymphocytes. The location of Tumor-infiltrating lymphocytes in TME may affect the progression of the tumor, in addition to altering the biology, density and type of the tumor. [12, 13]. Antigens of tumors are identified by B cells that also secrete specific antibodies of antitumor effect [10, 14].

In breast cancer, the antitumor activity of reactive mo-

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noclonal antibodies has been documented. Also, some data shows B cell response features like clonal expansion, isotype switching and somatic mutation, in (OSCC) [15, 16]. Many researches show B lymphocyte infiltration increases with the oral epithelium progression from dysplasia to carcinoma, while in OSCC the associated B lymphocyte prognostic impact of tumor has not been fully elucidated. (17). The extent to which all B cells develop suppressive functions in responses to environmental stimuli or possess distinct regulatory characteristics is uncertain.[17, 18].

Some models of tumors and chronic inflammation show the regulatory B Cells Immunosuppressive function [18]. The most associated regulatory B Cells cytokines are IL-10 in exacerbated autoimmune disease, tumor models and adoptive transfer. The Indication that regulatory B lymphocytes induce immunosuppression with regulatory T Cells by cross-talk in an IL-10-independent manner was done by Zhang et al. 2016 [19]. The infiltration of The role of B lymphocytes in solid carcinomas remains unclear due to their complex and sometimes opposing effects on tumor progression [20].

The present study sought to assess the expression of B lymphocytes in the tumor microenvironment of oral squamous cell carcinoma as well as their correlation with clinical outcomes.

2. Materials and Methods

2.1. Patients

This retrospective analysis comprised 40 tissue samples from patients diagnosed with OSCC at the Department of Oral Pathology, University of Baghdad College of Dentistry. The ethical committee approved the protocol of this investigation. The samples in this work were taken from tissue specimens collected during routine histopathological diagnosis. The diagnosis of oral squamous cell carcinoma (OSCC) was made by evaluating hematoxylene and eosin-stained tissue sections. Selected samples were collected from lesions that had complete surgical removal. None of the patients in our cohort had distant metastases. We obtained clinical data (gender, age, size, and tumor location) from medical records. Any Specimens with inadequate paraffin-embedded material or uncompleted clinicopathological data were excluded from the study.

2.2. Histopathologic evaluation

Whole tumor thickness slices stained with hematoxylin were utilized to histologically assess the tumor grade. Per WHO classification, they are categorized as (well , poorly, and moderately) differentiated.

2.3. Immunohistochemistry

This investigation produced sections from conventionally processed paraffin blocks, each four micrometers thick. Immunohistochemical staining was conducted with the peroxidase-labeled streptavidin-biotin method. Each segment underwent deparaffinization, rehydration in xylene, grading in ethanol, and further treatment with 3% hydrogen peroxide. Antigen retrieval was performed by immersing the slides in Tris-EDTA buffer (pH = 9.0) at 95°C and heating the sections for 20 minutes. Then for 10 minutes the sections were treated to quench endogenous peroxidase activity with H_2O2. After that, we block nonspecific binding by treated with protein block.

The slides were incubated with the following primary antibody: CD20 Rabbit monoclonal antibody (ab78237, Abcam, Cambridge, UK) for 1 hour at 37 °C and in humidified chamber overnight to detect B cells antibody at a dilution of 1: 150 for 24 hours.DAB detection kit (mouse and rabbit specific HRP/DAB) which was purchased from Abcam in Cambridge, United Kingdom (ab236466) was utilized in order to visualize the antibody-antigen complex. The sections were stained using Mayer's Mayer's hematoxylene, which was included in the kit. The tissue from the tonsils was employed as a positive control for the marker CD20.

Using the "whole slide imaging," all sections of OSCC were scanned. In all samples, tissue was examined, including the stromal area (tumor stroma) and intratumor area (Tumor Island). To evaluate the expression of CD20+ the mean values of positive cells in ten microscopic visual fields per section for each sample selected to the highest expression rate of CD20 showing, at 400x magnification. By two independent researchers, the CD20+ expression was examined by using a light microscope Olympus (Olympus, Tokyo, Japan). The immune-positive cells percentage was recorded semi-quantitatively.

2.4. Data analysis

The data were analyzed using SPSS version 26. The statistics are displayed as the mean, SD, and ranges. Categorical data are shown using frequency distributions and associated percentages. A two-tailed independent t-test and Analysis of Variance (ANOVA) were employed to compare the continuous variables. The receiver operating characteristic (ROC) curve was analyzed to predict the CD20+ score as a distinguishing factor between stromal and intratumoral areas. A P-value below 0.05 was deemed statistically significant.

3. Results

The age range in the present investigation was 22 to 75 years with a mean of 53.87 ± 11.2 years. We noticed that 52.5% of patients were aged ≥ 55 years; 57.5% of them were males; the most common site of lesion was the tongue (45%); the lesion was well differentiated in 45% of cases; staged I in 37.5%; and lymph nodes were involved in 12.5% of patients (Table 1).

In comparison between stromal and intratumoral region of lesions by mean of CD20+ score, it was significantly higher in stromal region (40.29% versus 19.63%, P= 0.001) as shown in Table 2. Representative images of intratumoral and stromal expression of CD20+B lymphocytes are shown in Figures 1 and 2.

A study of the ROC curve was performed to determine the CD20+ score as a predictor for distinguishing between

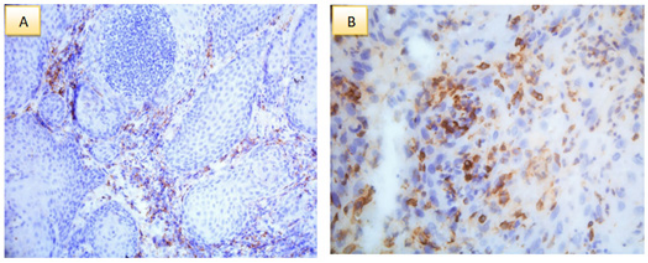

Fig. 1. A- Intratumoral CD20+ B- lymphocyte positive cells in OSCC-tissue specimens (200x, 400x)

Table 1. Distribution of study patients by clinical characteristics.

Variable	No. (n= 40)	Percentage (%)
Age (Year)		
< 55	19	47.5
≥ 55	21	52.5
Gender		
Male	23	57.5
Female	17	42.5
Site of lesion		
Tongue	18	45.0
Jaw	10	25.0
Buccal mucosa	7	17.5
Lip	3	7.5
Floor of the mouth	2	5.0
Grading (Differentiation)		
Well	18	45.0
Moderate	16	40.0
Poor	6	15.0
Stage		
I	15	37.5
II	14	35.0
III	4	10.0
IV	7	17.5
Lymph node (N) status		
Positive	5	12.5
Negative	35	87.5

Table 2. Comparison in mean of CD20+ score percentage by region of lesion

Region	CD20+ Score (%) Mean ± SD	P - Value
Stromal	40.29 ± 8.2	0.001
Intratumoral	19.63 ± 5.8	

stromal and intratumoral regions. The results are presented in Figure 3 and Table 3.

The cut point of the CD20+ percentage was 27%, so CD20+ score > 27% is predicator for stromal region as a large significant area upper the curve (AUC= 99%) indicating significant association between the CD20+ score and region. CD20+ score was 97.5% sensitive, 92.5% specific, and 95% accurate as a predictor for stromal region.

The comparison in mean of CD20+ score in stromal and intra-tumoral regions according to certain characteristics is shown in Table 4. In stromal and intra-tumoral regions, mean CD20+ score was significantly lower ($P < 0.05$) in well-differentiated lesions than in moderate and poorly differentiated lesions; and in staged I lesions than in staged II, III and IV lesions.

In stromal region, mean CD20+ score was significantly higher ($P = 0.01$) in lesions with lymph node involvement than in those without lymph node involvement.

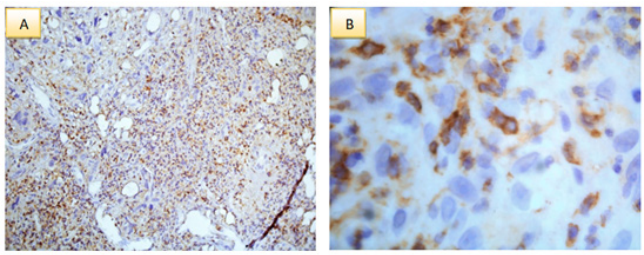

Fig. 2. (a,b) Stromal CD20+ B lymphocyte positives cells in OSCC tissue specimens (200x,400x).

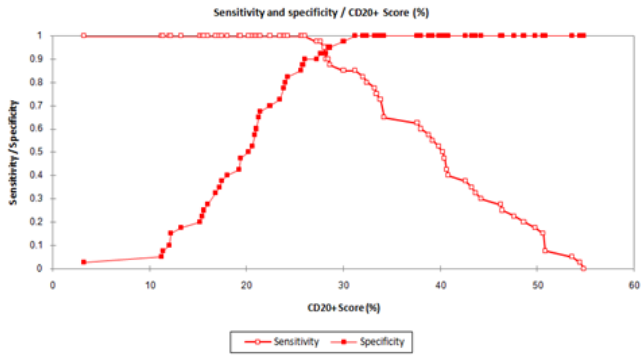

Fig. 3. ROC curve for CD20+ score as prediction for differentiation between stromal and intratumoral regions.

Table 3. Diagnostic accuracy of CD20+ score for prediction of differentiation between stromal and intratumoral regions.

CD20+ score (%)	Cut-off value	Sensitivity	Specificity	PPV	NPV	Accuracy
	27%	97.5%	92.5%	92.9%	97.4%	95%

Table 4. Comparison in mean of CD20+ score percentage in stromal region according to certain characteristics.

Variable	Stromal (%) Mean ± SD	P - Value	Intratumoral Mean ± SD	P - Value
Age (Years)				
≤ 55	41.78 ± 9.04	**0.281**	19.83 ± 6.9	**0.841**
> 55	38.93 ± 7.3		19.44 ± 4.8	
Gender				
Male	40.89 ± 8.6	**0.586**	20.56 ± 5.3	**0.256**
Female	39.47 ± 7.7		18.36 ± 6.4	
Site of lesion				
Tongue	42.73 ± 7.8	**0.366**	21.05 ± 4.3	**0.22**
Jaw	37.7 ± 8.6		16.82 ± 8.6	
Buccal mucosa	37.82 ± 7.9		18.4 ± 4.1	
Other	40.12 ± 8.7		21.84 ± 4.6	
Grading (Differentiation)				
Well	35.91 ± 6.8	**0.001**	18.34 ± 5.9	**0.03**
Moderate	41.01 ± 6.8		18.96 ± 5.6	
Poor	51.5 ± 2.5		25.26 ± 2.4	
Stage				
I	34.3 ± 7.1	**0.001**	16.28 ± 4.5	**0.007**
II	44.31 ± 6.4		22.78 ± 4.6	
III and IV	43.32 ± 7.2		20.18 ± 6.7	
Lymph node (N) status				
Positive	48.4 ± 5.1	**0.01**	19.32 ± 9.9	**0.941**
Negative	39.13 ± 7.9		19.67 ± 5.2	

4. Discussion

Oral squamous cell carcinoma is the predominant kind of head and neck squamous cell carcinoma, comprising over 90%, and is distinguished by its quick progression, high aggressiveness, and poor prognosis [21]. TME denotes a cellular milieu wherein stem cells reside within tumors or malignancies. The stem cells within the tumor possess self-renewal capabilities and promote carcinogenesis [22, 23]. The TME infiltration of Lymphocytes represents a tumor's host immune response. Up to now, many studies have examined the role of infiltrating T cells in the tumor micro-environmental [24]. However, the importance of B-lymphocyte cells in OSCC has received less attention, with studies showing inconsistent results [25].

Growing research indicates that infiltrating B cells can affect the tumor microenvironment, either promoting tumor growth or contributing to cytotoxic effects [26, 27]. B lymphocytes, as elements of the adaptive immune system, may play a more significant yet intricate function in oral squamous cell cancer [8]. Various techniques seek to elucidate the multifaceted functions of B cells in tumor immunology. Tumor-infiltrating B lymphocytes can release lymphotoxin, resulting in angiogenesis and the activation of STAT3 and NF-κB signaling, so facilitating tumor growth [7, 28]. In addition, presence of extracellular tumor vesicles can produce antibodies by activation of B cells and form the immune complexes and finally suppress the response of antitumor CD4+ and CD8+. However, TME cellular components determine the immune antitumor response in many cancers like gastric cancer which is characterized by high CD20+ B cell numbers that are considered as an independent predictor for excellent prognosis [29].

Erdag G. et al (2012) study on metastatic melanoma found that tumor-infiltrating B lymphocytes were identified as the second prognostic factor after CD8+ tumor-infiltrating lymphocyte [30]. Additionally, Knief et al (2016) reported in their study on the Adenocarcinoma of the Esophagogastric Junction high infiltrating of B lymphocyte and plasma cells, which correlates with prolonged overall survival [31].

Our study on CD20+ tumor-infiltrating B lymphocytes and their association with clinical outcomes in OSCC demonstrate that the CD20+ infiltration is significantly higher in the stromal region than in the intratumoral region. This finding can explain a potential differential role of CD20+ B lymphocytes depending on their localization relative to the tumor, where the higher presence in the stromal region may suggest an immune response or surveillance activity at the boundary of the tumor, potentially influencing the regulation of the local immunological milieu.

Shao et al., 2014 found an increasing in the number of B cells in the stromal region at the Hepatocellular carcinoma tumor margin that may associated with cancer progression [32].

Our investigation revealed a statistically significant association among stromal CD20+ B cells and lymph node metastases. The current finding aligns with prior studies on non-small cell lung carcinoma, breast carcinoma, and esophageal carcinoma. Research indicates that a higher prevalence of CD20+ B cells inside the tumor micro-environmental correlates with enhanced prognosis and survival rates [10, 33].

The studies conducted by Sales de Sá et al. and Z MA Mohammed et al. have shown that a significant presence of B cells is a reliable predictor of improved overall survival and suggests the presence of an immunologically

active, inflammatory TIME in surviving patients [34,35]. Taghavi et al., 2018 discovered a negative relationship between stromal CD20+ levels and the likelihood of metastatic spread to lymph nodes [2].

Conversely, Pretscher et al., 2009 observed increased intratumoral CD20+ numbers in metastatic tumors [36]. Additionally, a significant decrease in CD20+ cell infiltration was observed in well-differentiated OSCC in both stromal and intratumoral regions; this suggests that well-differentiated tumors have a more active or altered immune microenvironment. Nielsen et al. 2012 [37] finding improved that CD20+ B cells in poor differentiated serous ovarian cancer are found together with CD8+ T cells, leading to elevating the possibility of acting of B lymphocytes as antigen-presenting cells, which enables the cytolytic reaction of antitumoral T Cell [37].

Also, we found a significant decrease of CD20+ infiltration in stage I of both intratumoral and stromal areas, which may reflect a shift in immune cell distribution or activity as the tumor progresses. This finding disagrees with Distel et al., 2009 [38] who reported an increase in the number of CD20+ B cells in stage I of squamous cell carcinoma in the hypopharynx, which was associated with refined loco-regional control, while infiltration of CD20+ cells in advanced tumors, served as an indicator of poor prognosis. They suggested that CD20+ B cells have an important antitumor function in the initial stages of tumor progression by producing antibodies and antigen presentation [38]. On the other hand, Lundgren et al., in their study on ovarian epithelial tumors, disagree with our results, where they didn't find a significant relation between CD20+ B cell infiltration and prognosis [39].

Our study did not find any significant association of CD20+ B cell expression with other clinicopathological characteristics such as age, gender, or specific site of lesions within the stromal and intratumoral regions, proposed that the observed differences in CD20+ expression are more closely related to tumor differentiation, staging, and lymph node involvement rather than these demographic factors.

5. Conclusion

The findings indicate that CD20+ B cells significantly influence OSCC, particularly in relation to the tumor micro-environment and its progression. The increased infiltration of CD20+ B lymphocytes in stromal areas, particularly in cases with lymph node involvement, may represent an adaptive immune response or may be used as a prognostic marker to identify a potential aggressiveness or metastasis of OSCC. However, these results require further confirmation through confirmation in large, independent cohorts of OSCC. We suggest more studies are needed to differentiate between the "Tumor promoting" and "tumor inhibiting" effect of B-cell subsets in OSCC to develop more precise prognostic markers, where we can utilize some distinct markers that help to determine the role of B lymphocytes.

Limitations

A disadvantage of the present investigation was the small number of samples due to the confinement to excisional specimens from surgical neck dissections. Subsequent research should seek to corroborate those results in a more extensive patient group

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Conflict

No conflict of interest.

References

1. Bolesina N, Gatti G, López de Blanc S, Dhooge S, Rocha D, Fernandez E, Ferreyra R, Palla V, Grupe V, Morelatto R, Maccioni M (2022) Oral squamous cell carcinoma (OSCC) tumors from heavy alcohol consumers are associated with higher levels of TLR9 and a particular immunophenotype: Impact on patient survival. Front Immunol 5;13:941667. doi: 10.3389/fimmu.2022.941667.
2. Taghavi N, Mohsenifar Z, Baghban AA, Arjomandkhah A (2018) CD20+ Tumor Infiltrating B Lymphocyte in Oral Squamous Cell Carcinoma: Correlation with Clinicopathologic Characteristics and Heat Shock Protein 70 Expression. Patholog Res Int 4;2018:4810751. doi: 10.1155/2018/4810751.
3. Tandon P, Dadhich A, Saluja H, Bawane S, Sachdeva S(2017) The prevalence of squamous cell carcinoma in different sites of oral cavity at our Rural Health Care Centre in Loni, Maharashtra - a retrospective 10-year study. Contemp Oncol (Pozn)21(2):178-183. doi: 10.5114/wo.2017.68628.
4. Feller LL, Khammissa RR, Kramer BB, Lemmer JJ(2013) Oral squamous cell carcinoma in relation to field precancerisation: pathobiology. Cancer Cell Int 3;13(1):31. doi: 10.1186/1475-2867-13-31.
5. Li Q, Liu X, Wang D, Wang Y, Lu H, Wen S, Fang J, Cheng B, Wang Z(2020) Prognostic value of tertiary lymphoid structure and tumour infiltrating lymphocytes in oral squamous cell carcinoma. Int J Oral Sci 15;12(1):24. doi: 10.1038/s41368-020-00092-3.
6. Zhou C, Wu Y, Jiang L, Li Z, Diao P, Wang D, Zhang W, Liu L, Wang Y, Jiang H, Cheng J, Yang J(2018) Density and location of $CD3^+$ and $CD8^+$ tumor-infiltrating lymphocytes correlate with prognosis of oral squamous cell carcinoma. J Oral Pathol Med47(4):359-367. doi: 10.1111/jop.12698.
7. Suárez-Sánchez FJ, Lequerica-Fernández P, Rodrigo JP, Hermida-Prado F, Suárez-Canto J, Rodríguez-Santamarta T, Domínguez-Iglesias F, García-Pedrero JM, de Vicente JC (2021) Tumor-Infiltrating $CD20^+$ B Lymphocytes: Significance and Prognostic Implications in Oral Cancer Microenvironment. Cancers (Basel) 21;13(3):395. doi: 10.3390/cancers13030395.
8. Weledji EP, Nsagha DS, Enoworock G, Mouladje M (2-15) Familial hepatocellular carcinoma in an endemic area: two case reports. BMC Res Notes 8: 415. doi: 10.1186/s13104-015-1366-7.
9. Lund FE(2008) Cytokine-producing B lymphocytes-key regulators of immunity. Curr Opin Immunol 20(3):332-8. doi: 10.1016/j.coi.2008.03.003.
10. He Y, Qian H, Liu Y, Duan L, Li Y, Shi G (2014) The roles of regulatory B cells in cancer. J Immunol Res215471. doi: 10.1155/2014/215471.
11. Workel HH, Lubbers JM, Arnold R, Prins TM, van der Vlies P, de Lange K, Bosse T, van Gool IC, Eggink FA, Wouters MCA, Komdeur FL, van der Slikke EC, Creutzberg CL, Kol A, Plat A, Glaire M, Church DN, Nijman HW, de Bruyn M (2019) A Transcriptionally Distinct $CXCL13^+CD103^+CD8^+$ T-cell Population Is Associated with B-cell Recruitment and Neoantigen Load in Human

Cancer. Cancer Immunol Res 7(5):784-796. doi: 10.1158/2326-6066.CIR-18-0517.

12. Cho KA, Lee JK, Kim YH, Park M, Woo SY, Ryu KH (2017) Mesenchymal stem cells ameliorate B-cell-mediated immune responses and increase IL-10-expressing regulatory B cells in an EBI3-dependent manner. Cell Mol Immunol 2;14(11):895–908. doi: 10.1038/cmi.2016.59
13. Schmidt M, Micke P, Gehrmann M, Hengstler JG (2012) Immunoglobulin kappa chain as an immunologic biomarker of prognosis and chemotherapy response in solid tumors. Oncoimmunology 1;1(7):1156-1158. doi: 10.4161/onci.21653.
14. Olkhanud PB, Damdinsuren B, Bodogai M, Gress RE, Sen R, Wejksza K, Malchinkhuu E, Wersto RP, Biragyn A (2011) Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting CD4+ T cells to T-regulatory cells. Cancer Res 71(10):3505-15. doi: 10.1158/0008-5472.CAN-10-4316.
15. Garnelo M, Tan A, Her Z, Yeong J, Lim CJ, Chen J, Lim KH, Weber A, Chow P, Chung A, Ooi LL, Toh HC, Heikenwalder M, Ng IO, Nardin A, Chen Q, Abastado JP, Chew V(2017) Interaction between tumour-infiltrating B cells and T cells controls the progression of hepatocellular carcinoma. Gut 66(2):342-351. doi: 10.1136/gutjnl-2015-310814.
16. Quan H, Fang L, Pan H, Deng Z, Gao S, Liu O, Wang Y, Hu Y, Fang X, Yao Z, Guo F, Lu R, Xia K, Tang Z (2016) An adaptive immune response driven by mature, antigen-experienced T and B cells within the microenvironment of oral squamous cell carcinoma. Int J Cancer 138(12):2952-62. doi: 10.1002/ijc.30019.
17. Rosser EC, Blair PA, Mauri C (2014) Cellular targets of regulatory B cell-mediated suppression. Mol Immunol 62(2):296-304. doi: 10.1016/j.molimm.2014.01.014.
18. Vadasz Z, Haj T, Kessel A, Toubi E (2013) B-regulatory cells in autoimmunity and immune mediated inflammation. FEBS Lett 27;587(13):2074-8. doi: 10.1016/j.febslet.2013.05.023.
19. Zhang Y, Morgan R, Chen C, Cai Y, Clark E, Khan WN, Shin SU, Cho HM, Al Bayati A, Pimentel A, Rosenblatt JD (2016) Mammary-tumor-educated B cells acquire LAP/TGF-β and PD-L1 expression and suppress anti-tumor immune responses. Int Immunol 28(9):423-33. doi: 10.1093/intimm/dxw007.
20. Han S, Zhang C, Li Q, Dong J, Liu Y, Huang Y, Jiang T, Wu A(2014)Tumour-infiltrating CD4(+) and CD8(+) lymphocytes as predictors of clinical outcome in glioma. Br J Cancer 13;110(10):2560-8. doi: 10.1038/bjc.2014.162.
21. Caruntu A, Moraru L, Surcel M, Munteanu A, Costache DO, Tanase C, Constantin C, Scheau C, Neagu M, Caruntu C (2022) Persistent Changes of Peripheral Blood Lymphocyte Subsets in Patients with Oral Squamous Cell Carcinoma. Healthcare (Basel) 10;10(2):342. doi: 10.3390/healthcare10020342.
22. Ngamphaiboon N, Chureemas T, Siripoon T, Arsa L, Trachu N, Jiarpinitnun C, Pattaranutaporn P, Sirachainan E, Larbcharoensub N (2019) Characteristics and impact of programmed death-ligand 1 expression, CD8+ tumor-infiltrating lymphocytes, and p16 status in head and neck squamous cell carcinoma. Med Oncol 21;36(2):21. doi: 10.1007/s12032-018-1241-1.
23. Grivennikov SI, Greten FR, Karin M(2010) Immunity, inflammation, and cancer. Cell 19;140(6):883-99. doi: 10.1016/j.cell.2010.01.025.
24. Phulari RGS, Rathore RS, Shah AK, Agnani SS (2019) Neutrophil: Lymphocyte ratio and oral squamous cell carcinoma: A preliminary study. J Oral Maxillofac Pathol 23(1):78-81. doi: 10.4103/jomfp.JOMFP_160_17
25. Hadler-Olsen E, Wirsing AM (2019) Tissue-infiltrating immune cells as prognostic markers in oral squamous cell carcinoma: a systematic review and meta-analysis. Br J Cancer120(7):714-727. doi: 10.1038/s41416-019-0409-6.
26. Mandal R, Şenbabaoğlu Y, Desrichard A, Havel JJ, Dalin MG, Riaz N, Lee KW, Ganly I, Hakimi AA, Chan TA, Morris LG (2016) The head and neck cancer immune landscape and its immunotherapeutic implications. JCI Insight 20;1(17):e89829. doi: 10.1172/jci.insight.89829.
27. Thomas A, Smitha T, Rao K, Priya NS, Sheethal HS, Chitra S(2023) Expression of CD 20 B-Lymphocyte in oral epithelial dysplasia and oral squamous cell carcinoma: A comparative immunohistochemistry study. J Oral Maxillofac Pathol 27(2):323-327. doi: 10.4103/jomfp.jomfp_2_23.
28. Guo FF, Cui JW (2019)The Role of Tumor-Infiltrating B Cells in Tumor Immunity. J Oncol 24;2019:2592419. doi: 10.1155/2019/2592419.
29. Sakimura C, Tanaka H, Okuno T, Hiramatsu S, Muguruma K, Hirakawa K, Wanibuchi H, Ohira M. B cells in tertiary lymphoid structures are associated with favorable prognosis in gastric cancer. J Surg Res. 2017215:74-82. doi: 10.1016/j.jss.2017.03.033.
30. Erdag G, et al. (2012) Immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma. Cancer Res 72:1070–1080. [PubMed: 22266112].
31. Knief J , Reddemann K, Petrova E , Herhahn T, Wellner U And Thorns C (2016) High Density of Tumor-infiltrating B-Lymphocytes and Plasma Cells Signifies Prolonged Overall Survival in Adenocarcinoma of the Esophagogastric Junction. Anticancer Res36: 5339-5346. doi:10.21873/anticanres.11107.
32. Shao Y, Lo CM, Ling CC, Liu XB, Ng KT, Chu AC, Ma YY, Li CX, Fan ST, Man K (2014) Regulatory B cells accelerate hepatocellular carcinoma progression via CD40/CD154 signaling pathway. Cancer Lett 28;355(2):264-72. doi: 10.1016/j.canlet.2014.09.026.
33. Martinet L, Garrido I, Filleron T, Le Guellec S, Bellard E, Fournie JJ, Rochaix P, Girard JP (2011) Human solid tumors contain high endothelial venules: association with T- and B-lymphocyte infiltration and favorable prognosis in breast cancer. Cancer Res 1;71(17):5678-87. doi: 10.1158/0008-5472.CAN-11-0431.
34. Sales de Sá R, Miranda Galvis M, Mariz BALA, Leite AA, Schultz L, Almeida OP, Santos-Silva AR, Pinto CAL, Vargas PA, Gollob KJ, Kowalski LP (2021) Increased Tumor Immune Microenvironment CD3+ and CD20+ Lymphocytes Predict a Better Prognosis in Oral Tongue Squamous Cell Carcinoma. Front Cell Dev Biol 18;8:622161. doi: 10.3389/fcell.2020.622161.
35. Mohammed ZM, Going JJ, Edwards J, Elsberger B, McMillan DC (2013) The relationship between lymphocyte subsets and clinico-pathological determinants of survival in patients with primary operable invasive ductal breast cancer. Br J Cancer 17;109(6):1676-84. doi: 10.1038/bjc.2013.493.
36. Pretscher D, Distel LV, Grabenbauer GG, Wittlinger M, Buettner M, Niedobitek G(2009) Distribution of immune cells in head and neck cancer: CD8+ T-cells and CD20+ B-cells in metastatic lymph nodes are associated with favourable outcome in patients with oro- and hypopharyngeal carcinoma. BMC Cancer 22;9:292. doi: 10.1186/1471-2407-9-292.
37. Nielsen JS, Sahota RA, Milne K, Kost SE, Nesslinger NJ, Watson PH, Nelson BH(2012) CD20+ tumor-infiltrating lymphocytes have an atypical CD27- memory phenotype and together with CD8+ T cells promote favorable prognosis in ovarian cancer. Clin Cancer Res 15;18(12):3281-92. doi: 10.1158/1078-0432.CCR-12-0234.
38. Distel LV, Fickenscher R, Dietel K, Hung A, Iro H, Zenk J, Nkenke E, Büttner M, Niedobitek G, Grabenbauer GG(2009) Tumour infiltrating lymphocytes in squamous cell carcinoma of the oro- and hypopharynx: prognostic impact may depend on type of treatment and stage of disease. Oral Oncol 45(10):e167-74. doi:

10.1016/j.oraloncology.2009.05.640.
39. Lundgren S, Berntsson J, Nodin B, Micke P, Jirström K (2016) Prognostic impact of tumour-associated B cells and plasma cells in epithelial ovarian cancer. J Ovarian Res 6;9:21. doi: 10.1186/s13048-016-0232-0.