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Analysis of Serum Levels of Tumor Necrosis Factor Superfamily Member 4 (OX40L) in Naïve Patients of Oral Squamous Cell Carcinoma

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Abstract: Oral squamous cell carcinoma is one of the most common cancers in Pakistan, with a dismal prognosis despite the availability of multiple treatment modalities. Newer therapeutics areas are being explored to target advanced and metastatic tumors, and immunotherapy is one of them. Therapeutic agents based on the costimulatory immune molecules OX40 ("Tumor necrosis factor receptor superfamily member 4") and OX40L ("Tumor necrosis factor superfamily member") are studied in clinical trials for various cancers. However, scarce literature is available on the expression of OX40L in oropharyngeal squamous cell carcinoma (OSCC), and no data is available on its serum levels in naïve OSCC patients. Therefore, it is important to explore these molecules to enhance the basic level of knowledge and aid in the better utilization of these future therapeutic agents. Therefore, the purpose of this study is to measure serum levels of OX40L in newly diagnosed OSCC patients who have not undergone any prior treatment. After informed consent, blood was taken under aseptic conditions from 10 healthy individuals and 78 newly diagnosed OSCC patients. Serum was separated, and its levels were measured via ELISA. The analysis of data was performed by using SPSS version 25. The median serum levels of OX40L in controls were 220.9 (68.15) pg/ml, while in cases, it was 196.85 (107.75) pg/ml (p = 0.161). OX40L serum levels in well-differentiated tumors were 173.50 (68.15) pg/ml, moderately differentiated tumors showed 204.90 (90.30) pg/ml, and poorly differentiated tumors showed serum levels of 273.90 pg/ml (p = 0.201). Serum OX40L levels in early-stage (Stage I and Stage II) and late-stage (Stage III and Stage IV) were 208.25 (114.9) pg/ml and 194.5 (111.2) pg/ml, respectively (p = 0.143). The serum OX40L levels were insignificantly related to age, gender, habits, tumor sites, clinical staging, and histological grading. It is the first study that evaluated serum OX40L levels in naïve OSCC patients providing a baseline for future studies.

Keywords: OX40L, OX40 ligand, immunotherapy, oral squamous cell carcinoma.

初治口腔鳞癌患者血清肿瘤坏死因子超家族成员 4 (牛40) 水平分析

摘要：口腔鳞状细胞癌是巴基斯坦最常见的癌症之一，尽管有多种治疗方式，但预后不佳。正在探索新的治疗领域，以靶向晚期和转移性肿瘤，免疫疗法就是其中之一。在各种癌症的临床试验中研究了基于共刺激免疫分子牛40（“肿瘤坏死因子受体超家族成员4”）和牛40升（“肿瘤坏死因子超家族成员”）的治疗剂。然而，关于牛40升在口咽鳞状细胞癌(OSCC)
中表达的文献很少，并且没有关于其在初治OSCC患者中的血清水平的数据。因此，探索这些分子以提高基础知识水平并帮助更好地利用这些未来的治疗剂非常重要。因此，本研究的目的是测量未接受任何先前治疗的新诊断OSCC患者的血清牛40升水平。知情同意后，在无菌条件下从10名健康个体和78名新诊断的OSCC患者中抽取血液。分离血清，并通过酶联免疫吸附试验测量其水平。使用SPSS25版进行数据分析。对照组中牛40升的中位血清水平为2

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20.9（68.15）皮克/毫升，而在病例中为196.85（107.75）皮克/毫升（p=0.161）。高分化肿瘤中的牛40升血清水平为 173.50 (68.15) 皮克/毫升，中度分化肿瘤显示为 204.90 (90.30) 皮克/毫升，低分化肿瘤显示血清水平为 273.90皮克/毫升(p=0.201)。早期（I 期和 II 期）和晚期（III 期和 IV 期）的血清牛40升水平分别为 208.25 (114.9) 皮克/毫升和 194.5 (111.2)皮克/毫升(p=0.143)。血清牛40升水平与年龄、性别、生活习惯、肿瘤部位、临床分期和组织学分级无关。这是第一项评估初治OSCC患者血清牛40升水平的研究，为未来的研究提供了基线。

关键词：牛40、牛40 配体、免疫疗法、口腔鳞状细胞癌。

1. Introduction

Oral cancer is one of the prevalent cancers worldwide. According to cancer statistics, oral cavity and lip cancer are the most prevalent in the male gender, while in Pakistani females, it ranks second [1]. The most common malignancy of the head and neck region is oral squamous cell carcinoma (OSSC). Regardless of advancements in medicine, surgery, artificial intelligence utilization as bioinformatic tools, molecular biology, and diagnostic modalities, the prognosis remains dismal for OSCC. It has been the same 40–50% for the last two decades [2, 3]. Cancer immunotherapy is a new, emerging, and expanding field of therapeutics in which multiple immunotherapeutic agents are being devised and investigated through medical trials. Agents capable of blocking negative immune check points such as "cytotoxic T-lymphocyte–associated antigen 4" (CTLA-4) and "programmed death 1" (PD-1) have obtained FDA clearance for the treatment of metastatic and recurrent tumours. However, the success stories are only few and come with the cost of side effects [4].

Additional immune modulators such as costimulatory mediators, OX40 and OX40L, are being scrutinized for their antitumor action in monotherapy or combination therapy to reap more benefits. On OX40 (“Tumour Necrosis Factor Receptor Superfamily, member 4; TNFRSF4”) is expressed in activated T cells. OX40L (“Tumour necrosis factor superfamily, member 4; TNFSF4”), on the other hand, is primarily expressed in antigen-presenting and endothelial cells [5]. The current trials on OX40 agonists have yielded positive results in the form of immunity boost through increase in CD4 and CD8 T cells after treatment with them [6, 7]. Nevertheless, long-term outcomes are yet to be seen.

However, considering the fact that tumour cells' capacity to escape the immune system also includes their ability to alter the activities of immunological mediators, it is necessary to investigate the levels of expression of such immune mediators in cases of cancer. This will facilitate a better understanding of their therapeutic use. Scarce data is available on the expression of OX40L in cancers with conflicting results. OX40L has been studied in colorectal cancer and lung adenocarcinoma in tumour biopsies, primarily in tumour infiltrating lymphocytes (TILs) and tumour cells, as well as in serum [8-11]. However, to the best of our knowledge and from the literature search, no study has evaluated serum levels of OX40L in OSCC. This study aims to measure serum levels of OX40L in newly diagnosed OSCC patients who have not undergone any prior treatment.

2. Methods

2.1. Study Design

This is a cross-sectional study, and the ethical approval was taken from Ziauddin University, Karachi, Pakistan (ERC# 2410720ASBC). The demographic data such as age, gender, ethnicity, occupation, educational status was recorded. Moreover, clinical parameters such as sites of the tumor, habits of any substance abuse (smoking, alcohol, chewable tobacco products, etc.), histological grading, and clinical staging were noted.

2.2. Participants

The study involved 10 healthy controls and 78 newly diagnosed biopsy-confirmed OSCC patients without prior treatment. This research was conducted from December 2020 to July 2021. The exclusion criteria included autoimmune disorders and any malignancy other than OSCC.

2.3. Estimation of Serum Levels of OX40L

Following informed consent, a skilled phlebotomist drew 5 mL of blood by undertaking all required aseptic measures. The tube containing blood was centrifuged at 2000 revolutions per minute (RPM) for 15 minutes to separate serum. The separated serum was then decanted in Eppendorf tubes and saved at -80°C for further processing in the future. We carried out blood serum tests to check OX40L levels using the enzyme-linked

immunosorbent assay (ELISA) kit (SEA850Hu, Cloud Clone Corp., Wuhan, China). The experiment was performed according to the protocol provided by the manufacturer.

2.4. Statistical Analysis

Data was analyzed using version 25 of the IBM-SPSS statistical program (IBM Corp., Chicago, IL). The normality of the data was determined through the Shapiro–Wilk test. Categorical variables were expressed as frequencies and percentages. The continuous variables were expressed as mean ± SD and as median (interquartile range (IQR)). The comparison of medians for controls and cases and gender was performed by using the Mann–Whitney U test. The analysis for serum levels of OX40L in different habits, tumor sites, histological grades, and clinical stages was executed by the Kruskal–Wallis test, considering p value < 0.05 as statistically significant.

3. Results

3.1. Baseline Characteristics of the Study Population

This study comprised a total of 78 histologically confirmed cases of oral squamous cell carcinoma with no prior therapy, as well as 10 controls. The controls (n = 10) had a mean age of 425.58, whereas the OSCC patients had a mean age of 49.9113 years. Among the study population, there were 65 males (83%), while 13 (17%) were females. The common habits among our study group were smoking alone (30%), consuming more than one tobacco-based product (29.5%), and paan consumption alone (25.64%). The most common tumor site was buccal mucosa (64.1%), followed by lip (14.1%). The most frequent type of tumor, according to the histological grading, was well-differentiated tumors (n = 40, 51.28%), followed by moderately differentiated tumors (n = 37, 47.4%), and only one case of poorly differentiated tumors (n = 1, 1.28%). The OSCC patients were categorized into early-stage (Stage I and Stage II) and late-stage (Stage III and Stage IV). In the early stage, there were 21 patients (26.92%), and in the late stage, there were 57 (73.08%) patients.

The mean OX40L levels were 228.01 ± 49.86 pg/ml in controls and 217.61 ± 1276.30 pg/ml in OSCC patients. The median serum levels of OX40L were 220.9 (68.15) pg/ml in controls and 196.85 (107.75) pg/ml (p = 0.161) in cases. The serum levels of OX40L were evaluated based on age and gender. The serum levels of OX40L were 171.7 (120.35) pg/ml in patients less than 50 years of age, and in patients who were more than 50 years of age, the levels were 209.35 (88.75) pg/ml; this is not a statistically significant difference (p value = 0.16). The serum levels of OX40L were 200.7 (95.75) pg/ml in males and 213.5 (121.65) pg/ml in females and were found to not be significantly different (p value = 0.97).

3.2. Serum Levels of OX40 and OX40L Depending on Habits and Sites of Tumor of OSCC Patients

The serum OX40L levels were measured in OSCC patients in relation to their habits and were found to be non-significant (p value = 0.340). Likewise, the serum levels of OX40L were assessed in relation to different tumor locations of squamous cell carcinoma and were found to be non-significant (p value = 0.78), as depicted in Table 1.

Table 1 Serum levels of OX40L in OSCC patients depending on habits and site of the tumor

Serum levels of OX40L (pg/ml)				
	n	**Mean ± SD**	**Median (IQR)**	**p-value**
Habits				
Smoking	24	252.3 ± 170.7	210.35 (105.88)	0.340
Alcohol	4	220.55 ± 73.63	216.95(142.32)	
Paan	20	226.87 ± 124.23	206.7(80.80)	
Naswar	1	150.40	150.4	
>1 habit	23	183.85 ± 68.98	166.9 (123.30)	
Sites of Tumor				
Cheek	50	219.0 ± 136.92	190.25(108.73)	0.779
Tongue	8	196.63 ± 65.08	179.0 (130.35)	
Alveolus	5	201.97 ± 125.01	150.4(221.56)	
Lip	11	237.61 ± 150.4	215.8(92.90)	
Palate	4	206.72 ± 19.17	203.90 (36.35)	

3.3. The Serum Levels of OX40L According to the Histological Grades in OSCC

The OX40L serum levels were assessed in different histological grades of OSCC; well differentiated tumors revealed 173.50 (68.15) pg/ml, moderately differentiated ones showed levels of 204.90 (90.30) pg/ml, and there was only one case of poorly differentiated tumors, which showed serum levels of 273.90 pg/ml. There was no significant difference in OX40L serum levels between histological grades (p = 0.201).

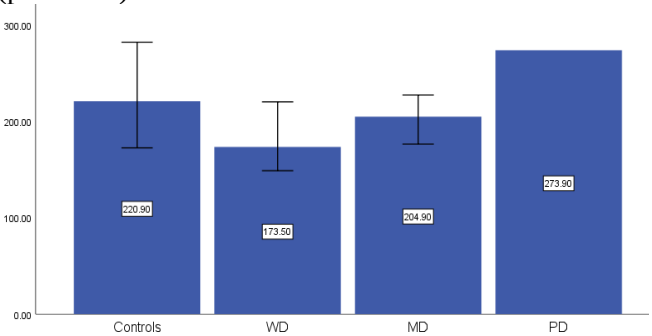

Fig. 1 Serum levels of OX40L in different histological grades of OSCC

Notes: The Kruskal–Wallis test revealed an overall significant p value of 0.203 (WD = well differentiated, MD = moderately differentiated, and PD = poorly differentiated)

3.4. Serum Levels of OX40 and OX40L in the Early and Late Stages of OSCC

According to the stage, the OSCC patients were categorized into early stage (Stage I and Stage II) and late stage (Stage III and Stage IV). ELISA was performed on 10 healthy individuals, 21 patients in the early stage, and 57 patients in the late stage. The OX40L level in healthy individuals (n = 10) was 220.9 (68.15) pg/ml, whereas in early-stage and late-stage patients, median serum levels of 208.25 (114.9) pg/ml and 194.5 (111.23) pg/ml were observed, respectively. There was a non-significant difference between the controls, early-stage patients, and late-stage patients (p = 0.143), as illustrated in Fig. 2.

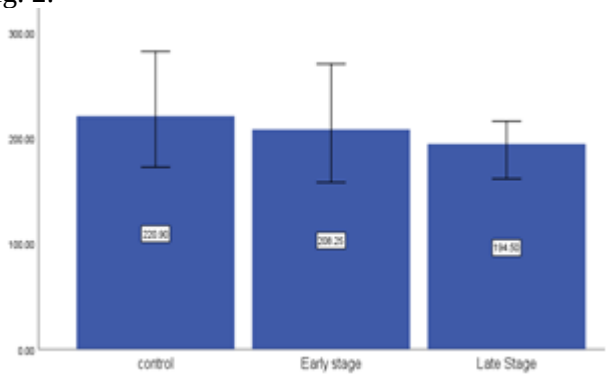

Fig. 2 Serum levels of OX40L (pg/ml) in the early and late stages of OSCC
Note: Kruskal Wallis test revealed an overall significant *p*-value of 0.143

4. Discussion

The tumor necrosis factor superfamily member 4 (OX40L) is a cognate ligand of the tumor necrosis factor receptor superfamily member 4 (OX40) and has an immune costimulatory role when the two interact, thus amplifying the immune response against threats. The expression of OX40L is diverse and has been reported in antigen-presenting cells (i.e., endothelial cells), activated T cells, and other immune cells [12]. OX40L is thought to be a potential target for enhancing antitumor immunity due to its interaction with OX40. Hence, multiple pharmaceutical agent trials are focusing on OX40 agonists, which can simulate the physiological role of OX40L. OX40L has been evaluated in asthma, atopic dermatitis, recurrent spontaneous abortion, and systemic sclerosis [13–16]. Research on the serum levels of OX40L in malignancies, on the other hand, is limited. The majority of studies on cancer have examined OX40L expression in biopsy samples. However, blood samples are a comparatively non-invasive alternative that can provide information not only about the state of the patient's body but also about the milieu of the tumor tissue [11]. To the best of our knowledge, no publications pertaining to serum OX40L levels in OSCC and their association with distinct histological grades and clinical stages (which is the foundation of cancer therapy decisions) have been identified.

The present study has demonstrated that the OX40L serum levels were decreased in patients with well differentiated tumors compared to controls and those with moderately differentiated tumors. Although poorly differentiated tumors were shown to lead to higher serum levels, there was only one such case in our sample, which may not be enough to provide conclusive evidence. The results of the present study showed that the OX40L serum levels decreased in a stage-dependent manner, although the difference was non-significant ($p \leq 0.143$). This is the first study to analyze OX40L levels in patients with naive oral squamous cell carcinoma. Overall, data on the serum levels of OX40L in malignancies are scarce. Although our results are non-significant, decreasing OX40L serum levels in the advanced stage may indicate a dysregulated immune status. Kashima et al. found that higher levels of OX40L were linked with a poor prognosis in advanced lung adenocarcinoma (n = 56) and concluded that higher serum levels may reflect an exhausted immune state against tumors in advanced stages [8]. There is conflicting literature on the predictive value of OX40L expression depending on the type of cancer. In bladder cancer, OX40L overexpression has been associated with a higher risk of recurrence [17]; however, longer progression-free survival in glioblastoma was observed with OX40L overexpression [18]. Furthermore, OX40L has been reported to be overexpressed in 74% of head and neck cancer (HNC) patients associated with poor prognoses [11]. Another study reported the overexpression of OX40L in the mesenchymal subtype of oral squamous cell carcinoma, indicating an altered immune status [19]. Nevertheless, the levels of OX40L may be affected by the state of inflammation in OSCC patients, and our results may differ from those of comparable studies due to the use of a different technique (ELISA).

Currently, OX40L mRNA transfected into dendritic cells, genetically engineered surfaces expressing OX40L tumor cells, and OX40L-Fc fusion proteins have shown translational relevance in animal and in vitro models by enhancing antitumor activity [20–22]. However, clinical trials on OX40/OX40L-based therapeutic agents are needed to establish the role of OX40L in cancer treatment [23].

We acknowledge that our study's main limitation was the small sample size of 78 OSCC patients. We anticipate that measuring OX40L in OSCC patients' serum, assessing its expression at the mRNA level in blood, and comparing levels in post-treatment patients can provide insights into new immunotherapies for OSCC. Longitudinal studies with larger sample sizes or case-control studies will be required in the future to confirm our findings and establish the utility of OX40L as a predictive biomarker for OSCC therapy.

5. Conclusions

The present study revealed that the serum levels of OX40L were not significant in naive OSCC patients in relation to different clinicopathological features. However, decreased levels in advanced stages may indicate a dysregulated immune status in OSCC patients. This information may aid in understanding the role of OX40L in patients newly diagnosed with OSCC, as no study on the serum levels of OX40L in naive OSCC patients is available to provide baseline knowledge. The main limitations of our work are that the OX40L levels in post-treatment patients were not analyzed and that the sample size was too small. In this study, we evaluated only the levels of OX40L, a secondary costimulator; however, cancer progresses with the interplay of multiple mediators. Other upstream and downstream mediators should be evaluated for a better understanding of the mechanisms involved in OSCC. In order to corroborate our findings regarding the role of OX40L in OSCC, this study needs to be replicated with a larger sample along with further clinical, molecular-level, and interventional investigations to elucidate the role of OX40/OX40L expression in OSCC patients and support the rationale to target these molecules as markers of immunotherapy.

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References

[1] ANWAR N., PERVEZ S., CHUNDRIGER Q., AWAN S., MOATTER T., and ALI T. S. Oral cancer: Clinicopathological features and associated risk factors in a high-risk population presenting to a major tertiary care center in Pakistan. *PLoS ONE*, 2020, 15: e0236359. https://doi.org/10.1371/journal.pone.0236359

[2] BRENNER C. Applications of Bioinformatics in Cancer. *Cancers*, 2019, 11: 1630. https://doi.org/10.3390/cancers11111630

[3] MAJUMDAR B., PATIL S., SARODE S. C., SARODE G. S., and RAO R. S. Clinico-pathological prognosticators in oral squamous cell carcinoma. *Translational Research in Oral Oncology*, 2017, 2: 1–14. https://doi.org/10.1177/2057178X17738912

[4] VENTOLA C. L. Cancer Immunotherapy, Part 3: Challenges and Future Trends. *Pharmacy and Therapeutics*, 2017, 42(8): 514–521. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5521300/

[5] DENG J., ZHAO S., ZHANG X., JIA K., WANG H., ZHOU C., and HE Y. OX40 (CD134) and OX40 ligand, important immune checkpoints in cancer. *OncoTargets and Therapy*, 2019, 12: 7347–7353. https://doi.org/10.2147/OTT.S214211

[6] DUHEN R., BALLESTEROS-MERINO C., FRYE A. K., TRAN E., RAJAMANICKAM V., CHANG S. C., KOGUCHI Y., BIFULCO C. B., BERNARD B., LEIDNER R. S., CURTI B. D., FOX B. A., URBA W. J., BELL R. B., and WEINBERG A. D. Neoadjuvant anti-OX40 (MEDI6469) therapy in patients with head and neck squamous cell carcinoma activates and expands antigen-specific tumor-infiltrating T cells. *Nature Communications*, 2021, 12: 1047. https://doi.org/10.1038/s41467-021-21383-1

[7] BELL R. B., LEIDNER R. S., CRITTENDEN M. R., CURTI B. D., FENG Z., MONTLER R., GOUGH M. J., FOX B. A., WEINBERG A. D., and URBA W. J. OX40 signaling in head and neck squamous cell carcinoma: Overcoming immunosuppression in the tumor microenvironment. *Oral Oncology*, 2016, 52: 1–10. https://doi.org/10.1016/j.oraloncology.2015.11.009

[8] KASHIMA J., OKUMA Y., HOSOMI Y., and HISHIMA T. High Serum OX40 and OX40 Ligand (OX40L) Levels Correlate with Reduced Survival in Patients with Advanced Lung Adenocarcinoma. *Oncology*, 2020, 98: 303–310. https://doi.org/10.1159/000505975

[9] SAWADA R., ARAI Y., SAGAWA Y., NAGATA Y., NISHIMURA T., NOGUCHI M., AMANO K., ARIHIRO S., SARUTA M., and HOMMA S. High blood levels of soluble OX40 (CD134), an immune costimulatory molecule, indicate reduced survival in patients with advanced colorectal cancer. *Oncology Reports*, 2019, 42: 2057–2064. https://doi.org/10.3892/or.2019.7304

[10] MONTLER R., BELL R. B., THALHOFER C., LEIDNER R., FENG Z., FOX B. A., CHENG A. C., BUI T. G., TUCKER C., HOEN H., and WEINBERG A. OX40, PD-1 and CTLA-4 are selectively expressed on tumor-infiltrating T cells in head and neck cancer. *Clinical & Translational Immunology*, 2016, 5: e70. https://doi.org/10.1038/cti.2016.16

[11] LECERF C., KAMAL M., VACHER S., CHEMLALI W., SCHNITZLER A., MOREL C., DUBOT C., JEANNOT E., MESEURE D., KLIJANIENKO J., MARIANI O., BORCOMAN E., CALUGARU V., BADOIS N., CHILLES A., LESNIK M., KRHILI S., CHOUSSY O., HOFFMANN C., PIAGGIO E., BIECHE I., and LE TOURNEAU C. Immune gene expression in head and neck squamous cell carcinoma patients. *European Journal of Cancer*, 2019, 121: 210–223. https://doi.org/10.1016/j.ejca.2019.08.028

[12] ALVES COSTA SILVA C., FACCHINETTI F., ROUTY B., and DEROSA L. New pathways in immune stimulation: targeting OX40. *ESMO Open*, 2020, 5: e000573. https://doi.org/10.1136/esmoopen-2019-000573

[13] MA S.-L., & ZHANG L. Elevated serum OX40L is a biomarker for identifying corticosteroid resistance in pediatric asthmatic patients. *BMC Pulmonary Medicine*, 2019, 19: 66. https://doi.org/10.1186/s12890-019-0819-5

[14] FURUE M., & FURUE M. OX40L–OX40 Signaling in Atopic Dermatitis. *Journal of Clinical Medicine*, 2021, 10(12): 2578. https://doi.org/10.3390/jcm10122578

[15] RAHMANI F., HADINEDOUSHAN H., and GHASEMI N. Relative Expression of OX40, OX40L mRNA, and OX40L Serum Levels in Women with Recurrent Spontaneous Abortion. *Immunological Investigations*, 2019, 48(5): 480–489.

https://doi.org/10.1080/08820139.2019.1567530
[16] DUFFUS K., LÓPEZ-ISAC E., TERUEL M., SIMEÓN C. P., CARRERIA P., ORTEGO-CENTENO N., VICENTE E., WORTHINGTON J., HERRICK A. L., and MARTIN J. Association of TNFSF4 (OX40L) polymorphisms with systemic sclerosis–related calcinosis. *Rheumatology*, 2019, 58: 1299–1301. https://doi.org/10.1093/rheumatology/kez015
[17] LE GOUX C., VACHER S., PIGNOT G., SIBONY M., BARRY DELONGCHAMPS N., TERRIS B., PIAGGIO E., ZERBIB M., DAMOTTE D., and BIECHE I. mRNA Expression levels of genes involved in antitumor immunity: Identification of a 3-gene signature associated with prognosis of muscle-invasive bladder cancer. *OncoImmunology*, 2017, 6(11): e1358330. https://doi.org/10.1080/2162402X.2017.1358330
[18] SHIBAHARA I., SAITO R., ZHANG R., CHONAN M., SHOJI T., KANAMORI M., SONODA Y., KUMABE T., KANEHIRA M., KIKUCHI T., SO T., WATANABE T., TAKAHASHI H., IWABUCHI E., TANAKA Y., SHIBAHARA Y., SASANO H., ISHII N., and TOMINAGA T. OX40 ligand expressed in glioblastoma modulates adaptive immunity depending on the microenvironment: a clue for successful immunotherapy. *Molecular Cancer*, 2015, 14: 41. https://doi.org/10.1186/s12943-015-0307-3
[19] ZHANG S. Y., REN X. Y., WANG C. Y., CHEN X. J., CAO R. Y., LIU Q., PAN X., ZHOU J. Y., ZHANG W. L., TANG X. R., CHENG B., and WU T. Comprehensive Characterization of Immune Landscape Based on Epithelial-Mesenchymal Transition Signature in OSCC: Implication for Prognosis and Immunotherapy. *Frontiers in Oncology*, 2021, 11: 587862. https://doi.org/10.3389/fonc.2021.587862
[20] DANNULL J., NAIR S., SU Z., BOCZKOWSKI D., DEBECK C., YANG B., GILBOA E., and VIEWEG J. Enhancing the immunostimulatory function of dendritic cells by transfection with mRNA encoding OX40 ligand. *Blood*, 2005, 105: 3206–3213. https://doi.org/10.1182/blood-2004-10-3944
[21] REUTER D., STAEGE M. S., KÜHNÖL C. D., and FÖLL J. Immunostimulation by OX40 Ligand Transgenic Ewing Sarcoma Cells. *Frontiers in Oncology*, 2015, 5: 242. https://doi.org/10.3389/fonc.2015.00242
[22] ASPESLAGH S., POSTEL-VINAY S., RUSAKIEWICZ S., SORIA J.-C., ZITVOGEL L., and MARABELLE A. Rationale for anti-OX40 cancer immunotherapy. *European Journal of Cancer*, 2016, 52: 50–66. https://doi.org/10.1016/j.ejca.2015.08.021
[23] SANI A. I., ZIL-E-RUBAB, USMAN S., AHMED S. Z., and HOSEIN M. Role of OX40 and its ligand as costimulatory modulators in cancer immunotherapy. *AIMS Molecular Science*, 2021, 8: 161–173. https://doi.org/10.3934/molsci.2021012

参考文:

[1] ANWAR N.、PERVEZ S.、CHUNDRIGER Q.、AWAN S.、MOATTER T. 和 ALI T. S. 口腔癌：就诊于巴基斯坦主要三级医疗中心的高危人群的临床病理特征和相关危险因素. 公共科学图书馆一, 2020, 15: e0236359。 https://doi.org/10.1371/journal.pone.0236359
[2] BRENNER C. 生物信息学在癌症中的应用。癌症，2019，11：1630。https://doi.org/10.3390/cancers11111630
[3] MAJUMDAR B., PATIL S., SARODE S. C., SARODE G. S., 和 RAO R. S. 口腔鳞状细胞癌的临床病理预后指标。口腔肿瘤学的转化研究，2017，2：1-14。 https://doi.org/10.1177/2057178X17738912
[4] VENTOLA C. L. 癌症免疫疗法，第 3 部分：挑战和未来趋势。药学和治疗学，2017，42（8）：514–521。 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5521300/
[5] DENG J., ZHAO S., ZHANG X., JIA K., WANG H., ZHOU C., 和HE Y. 牛40 (光盘134) 和牛40 配体，癌症中的重要免疫检查点。肿瘤靶点和治疗, 2019, 12: 7347–7353。 https://doi.org/10.2147/OTT.S214211
[6] DUHEN R., BALLESTEROS-MERINO C., FRYE A. K., TRAN E., RAJAMANICKAM V., CHANG S. C., KOGUCHI Y., BIFULCO C. B., BERNARD B., LEIDNER R. S., CURTI B. D., FOX B. A., URBA W. J., BELL R. B., 和 WEINBERG A. D. 新辅助抗牛40 (医疗器械6469) 治疗头颈部鳞状细胞癌患者可激活和扩增抗原特异性肿瘤浸润吨细胞。自然通讯，2021，12：1047。https://doi.org/10.1038/s41467-021-21383-1
[7] BELL R. B.、LEIDNER R. S.、CRITTENDEN M. R.、CURTI B. D.、FENG Z.、MONTLER R.、GOUGH M. J.、FOX B. A.、WEINBERG A. D., 和 URBA W. J. 牛40 在头颈部鳞状细胞癌中的信号传导：克服肿瘤中的免疫抑制微环境。口腔肿瘤学，2016，52：1-10。 https://doi.org/10.1016/j.oraloncology.2015.11.009
[8] KASHIMA J.、OKUMA Y.、HOSOMI Y. 和 HISHIMA T. 高血清牛40 和牛40 配体 (牛40升) 水平与晚期肺腺癌患者的存活率降低相关。肿瘤学，2020，98：303-310。 https://doi.org/10.1159/000505975
[9] SAWADA R.、ARAI Y.、SAGAWA Y.、NAGATA Y.、NISHIMURA T.、NOGUCHI M.、AMANO K.、ARIHIRO S.、SARUTA M. 和 HOMMA S. 可溶性牛40 的高血液水平（光盘134）是一种免疫共刺激分子，表明晚期结直肠癌患者的存活率降低。肿瘤学报告，2019，42：2057-2064。 https://doi.org/10.3892/or.2019.7304
[10] MONTLER R.、BELL R. B.、THALHOFER C.、LEIDNER R.、FENG Z.、FOX B. A.、CHENG A. C.、BUI T. G.、TUCKER C.、HOEN H. 和 WEINBERG A. 牛40、PD-1 和 CTLA-4 在头颈癌中的肿瘤浸润吨细胞上选择性表达。临床与转化免疫学，2016，5：e70。 https://doi.org/10.1038/cti.2016.16
[11] LECERF C., KAMAL M., VACHER S., CHEMLALI W., SCHNITZLER A., MOREL C., DUBOT C., JEANNOT E., MESEURE D., KLIJANIENKO J., MARIANI O., BORCOMAN E. , CALUGARU V., BADOIS N., CHILLES A., LESNIK M., KRHILI S., CHOUSSY O., HOFFMANN C., PIAGGIO E., BIECHE I., 和LE TOURNEAU C. 头颈部鳞状细胞的免疫基因表达细胞癌患者。欧洲癌症

杂志，2019，121：210-223。
https://doi.org/10.1016/j.ejca.2019.08.028
[12] ALVES COSTA SILVA C.、FACCHINETTI F.、ROUTY B. 和 DEROSA L. 免疫刺激的新途径：靶向牛40。埃斯莫公开赛，2020，5：e000573。 https://doi.org/10.1136/esmoopen-2019-000573
[13] MA S.-L., & ZHANG L. 升高的血清牛40升是识别儿童哮喘患者皮质类固醇抵抗的生物标志物。BMC
肺医学，2019，19：66。https://doi.org/10.1186/s12890-019-0819-5
[14] FURUE M., & FURUE M. 牛40升–牛40特应性皮炎中的信号传导。临床医学杂志，2021，10(12): 2578. https://doi.org/10.3390/jcm10122578
[15] RAHMANI F.、HADINEDOUSHAN H. 和 GHASEMI N. 牛40、牛40升mRNA和牛40升血清水平在复发性自然流产妇女中的相对表达。免疫学调查，2019，48(5)：480–489。
https://doi.org/10.1080/08820139.2019.1567530
[16] DUFFUS K.、LÓPEZ-ISAC E.、TERUEL M.、SIMEÓN C. P.、CARRERIA P.、ORTEGO-CENTENO N.、VICENTE E.、WORTHINGTON J.、HERRICK A. L., 和 MARTIN J. 肿瘤坏死因子顺丰 4 协会 (牛40升)
与系统性硬化症相关的钙质沉着症的多态性。风湿病学，2019，58：1299-1301。
https://doi.org/10.1093/rheumatology/kez015
[17] LE GOUX C.、VACHER S.、PIGNOT G.、SIBONY M.、BARRY DELONGCHAMPS N.、TERRIS B.、PIAGGIO E.、ZERBIB M.、DAMOTTE D. 和 BIECHE I. 基因的 mRNA
表达水平参与抗肿瘤免疫：鉴定与肌肉浸润性膀胱癌预后相关的 3
基因特征。肿瘤免疫学，2017，6(11)：e1358330。
https://doi.org/10.1080/2162402X.2017.1358330
[18] SHIBAHARA I.、SAITO R.、ZHANG R.、CHONAN M.、SHOJI T.、KANAMORI M.、园田 Y.、KUMABE T.、KANEHIRA M.、KIKUCHI T.、SO T.、WATANABE T. 、TAKAHASHI H.、IWABUCHI E.、TANAKA Y.、SHIBAHARA Y.、SASANO H.、ISHII N. 和 TOMINAGA T. 胶质母细胞瘤中表达的牛40
配体根据微环境调节适应性免疫：成功免疫治疗的线索。分子癌症，2015，14：41。https://doi.org/10.1186/s12943-015-0307-3
[19] 张锡, 任喜, 王 C. Y., CHEN X. J., CAO R. Y., LIU Q., PAN X., ZHOU J. Y., ZHANG W. L., TANG X. R., CHENG B., and WU T. 综合表征免疫景观基于OSCC中的上皮-
间质转化特征：对预后和免疫治疗的影响。肿瘤学前沿，2021，11：587862。https://doi.org/10.3389/fonc.2021.587862
[20] DANNULL J.、NAIR S.、SU Z.、BOCZKOWSKI D.、DEBECK C.、YANG B.、GILBOA E. 和 VIEWEG J. 通过转染编码牛40 配体的 mRNA
增强树突细胞的免疫刺激功能。血液，2005，105：3206-3213。 https://doi.org/10.1182/blood-2004-10-3944
[21] REUTER D.、STAEGE M. S.、KÜHNÖL C. D. 和 FÖLL J. 牛40
配体转基因尤文肉瘤细胞的免疫刺激。肿瘤学前沿，2015，5：242。https://doi.org/10.3389/fonc.2015.00242
[22] ASPESLAGH S.、POSTEL-VINAY S.、RUSAKIEWICZ S.、SORIA J.-C.、ZITVOGEL L. 和 MARABELLE A. 抗牛40
癌症免疫疗法的基本原理。欧洲癌症杂志，2016，52：50-66。 https://doi.org/10.1016/j.ejca.2015.08.021
[23] SANI A. I.、ZIL-E-RUBAB、USMAN S.、AHMED S. Z. 和 HOSEIN M. 牛40
及其配体在癌症免疫治疗中作为共刺激调节剂的作用。目标管理系统分子科学，2021，8：161-173。https://doi.org/10.3934/molsci.2021012