Research Article

Cem Horozoglu, Dilara Sonmez, Ozlem Kucukhuseyin, Seyda Demirkol, Mehmet Tolgahan Hakan, Soykan Arikan and İlhan Yaylim*

The importance of sPD-1, sOX40L and sGITR in terms of clinicopathology and histopathology in gastric cancer

sPD-1, sOX40L ve sGITR'nin gastrik kanserde klinikopatolojik ve histopatolojik açıdan önemi

https://doi.org/10.1515/tjb-2020-0075
Received February 18, 2020; accepted July 14, 2020; published online June 21, 2021

Abstract

Objectives: Immunoregulatory GITR, OX40L and PD-1 molecules play an active role in the regulation of tumor microenvironment and have been shown in many cancer types. However, there are limited studies of these molecules in terms of gastric cancer. In our study, we aimed to determine the histopathological significance of sPD-1, sOX40L and sGITR levels in gastric cancer cases.

Methods: Serum samples were obtained from peripheral blood samples of 20 gastric cancer and 34 healthy controls and candidate biomarker levels were determined by ELISA.
Results: sGITR level was found to be 48.28 ± 2.66 pg/mL in the gastric cancers and 39.19 ± 2.01 pg/mL in the controls (p=0.014). sOX40L level was 6.13 ± 0.64 pg/mL in the patients and 8.68 ± 0.99 pg/mL in the control, and the sOX40L level was found to be 1.41 times higher in the control group compared to the patients (p=0.033). sGITR levels of signet-ring cell carcinoma were found to be higher than other adenocarcinomas (p=0.04). There was no difference between groups in sPD-1 (p=0.490).
Conclusions: We suggest that sGITR, sOX40L and molecules may play a role in tumor microenvironment regulation and histological type in gastric cancer.

Keywords: gastric cancer; sGITR; sOX40L; sPD-1; signet-ring cell carcinoma.

ÖZ

Giriş: İmmünoregülatör GITR, OX40L ve PD-1 molekülleri, tümör mikro ortamının düzenlenmesinde aktif bir rol oynar ve birçok kanser tipinde tanımlanmıştır. Buna karşın, mide kanseri açısından bu moleküllerle ilgili sınırlı sayıda çalışma vardır.
Amaç: Çalışmamızda mide kanseri olgularında sPD-1, sOX40L ve sGITR düzeylerinin histopatolojik önemini belirlemeyi amaçladık.
Gereç ve Yöntemler: Serum örnekleri 20 mide kanseri ve 34 sağlıklı kontrolün periferik kan örneklerinden elde edildi ve aday biyobelirteçlerin seviyeleri ELISA ile saptandı.
Bulgular: sGITR düzeyi mide kanserli olgularda 48.28 ± 2.66 pg/mL ve control grubunda ise 39.19 ± 2.01 pg/

***Corresponding author: İlhan Yaylim,** Department of Molecular Medicine, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Vakif Gureba Cad, Sehremini-Fatih 34093 Istanbul, Turkey, Phone: +90 2124142000, Fax: +90 2125324171,
E-mail: ilhanyaylim@gmail.com. https://orcid.org/0000-0001-8998-2028
Cem Horozoglu, Department of Medical Biochemistry, Faculty of Medicine, Biruni University, Istanbul, Turkey,
E-mail: cem_horozoglu@hotmail.com
Dilara Sonmez, Ozlem Kucukhuseyin and Mehmet Tolgahan Hakan, Department of Molecular Medicine, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey,
E-mail: dilarasnmz@hotmail.com (D. Sonmez), a_ergen@yahoo.com (O. Kucukhuseyin), mthakan@istanbul.edu.tr (M.T. Hakan)
Seyda Demirkol, Department of Molecular Medicine, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey; and Department of Molecular Biology, Faculty of Engineering and Natural Science, Biruni University, Istanbul, Turkey,
E-mail: seyda.erc@gmail.com
Soykan Arikan, Department of General Surgery, Istanbul Training and Research Hospital, Istanbul, Turkey,
E-mail: soykanarikan@yahoo.com

Open Access. © 2020 Cem Horozoglu et al., published by De Gruyter. This work is licensed under the Creative Commons Attribution 4.0 International License.

mL olarak tespit edildi (p = 0.014). sOX40 L düzeyi hastalarda 6.13 ± 0.64 pg/mL tespit edilirken, kontrolde 8.68 ± 0.99 pg/m olarak tespit edilmiştir. sOX40 L düzeyinin kontrol grubuna göre 1,41 kat yüksek olduğu saptanmıştır (p = 0.033). Taşlı yüzük hücreli karsinomlu olguların sGITR düzeylerinin diğer adenokarsinomalı gruptan yüksek olduğu tespit edilmiştir (p = 0.04). sPD-1düzeyi açısından ise gruplar arasında fark tespit edilmemiştir (p = 0.490).

Anahtar Kelimeler: sGITR; sOX40L; sPD-1; Mide kanseri; taşlı yüzük hücreli karsinom.

Introduction

Members of the tumor necrosis factor receptor (TNFR) superfamily and glucocorticoid-induced tumor necrosis factor receptor family play a critical role in many types of cancer. GITR, CD134 (OX40) and CD137 (4-1BB) which are the members of these families contribute to the activation, proliferation, differentiation and cell death processes of both tumor and immune system cells [1, 2].

As a costimulatory receptor after multiple stimulation, GITR was upregulated on the surface of the regulator T cells (Treg, $CD4^+CD25^+Foxp3^+$) and the effector T cells ($CD4^+$ and $CD8^+$ T cells(Teff)) and also Natural Killers (NKs) for approximately 24 h to stimulate T cell activation, survival, improved effector function, or neutralize the suppression of the T cell.

According to this, in mature Tregs, FOXP3 strongly increases GITR expression and is subject to intrinsic regulation with canonical NFkB signaling. Besides, the increase in number in Teff either suppresses the proliferation of Treg by a negative feed-back mechanism or by producing IL-2 [1–5].

OX40 was found to have functional roles in cancer studies such as T cell memory, proliferation, antitumor activity, overcoming immunosuppression. OX40 has been shown to be specifically expressed in $CD4^+$ and $CD8^+$ T cells as well as in activated T cells, memory cells, NKs and neutrophils. The ligand of this costimulatory receptor, OX40L, is expressed in dendritic cells (DCs), macrophages, B cells as well as in the case of inflammatory regions. It was reported that the crosstalk between OX40/OX40L activates cell survival genes in which an important signaling for the long-term survival of $CD4^+$ T cells [6, 7].

As well-known that another tumor microenvironment regulator and immunoregulatory molecules programmed cell death protein 1 (PD-1), a member of the CD28 family and expressed in peripheral tissues, T lymphocytes, B lymphocytes, DCs, macrophages, NK and B lymphocytes [8–10]. The engagement of PD-1 with its ligand, PD-L1, plays a role in inhibiting T cell activation and proliferation. Additionally, it reduces the cytokine production which in turn results in negative or poor immune response especially through $CD8^+$ T cells as well [8, 11]. Actually, recent reports implied that the effects of increased PD-1/PD-L1 expression in tumor tissue and tumor-infiltrating lymphocytes on microsatellite instability carries high mortality risks [12, 13].

Clinical, experimental and functional studies in many different cancer types have been described in terms of immune molecules, which are thought to have a functional role in T cell activation and regulation of other cells and elements in the tumor microenvironment and immune response. However, according to our knowledge clinical and histopathological studies are very restricted in terms of gastric cancers related to sPD-1, sOX40L and sGITR which have been found to play a role in apoptosis, anti-tumor response, regulation of tumor microenvironment and T cell-mediated immune response, especially in many different tissue-specific cancer types. Thus, in the present study we aimed to investigate the differences between the levels of sPD-1, sOX40L and sGITR with histopathological and tumor type in gastric cancers.

Materials and methods

Sample selection

The blood samples of 20 gastric cancer patients and 34 healthy volunteers as control group which were followed by General Surgery Clinic of Istanbul Training and Research Hospital were included in this study. For histopathological evaluation of resected tumor specimens; tissue processing, tissue blocking/embedding and microtomy procedures were performed and stained with hematoxylin-eosin. Microscopic evaluations were assessed according to AJCC eighth TNM staging for gastric cancers.

Detection of sOX40L, sGITR and sPD-1 levels

sOX40L, sGITR and sPD1 levels in serum samples obtained from peripheral blood were determined using ELISA assay kit (Abbkin, China). Briefly, serum samples (GITR; 1/80, PD-1; 1/10, OX40L; 1/5 dilution) and standards were added to the 96-well plate and incubated 45 min at 37 °C. After incubation washing 5 times with washing buffer (250 µL), followed by incubation 30 min at 37 °C with HRP-conjugated streptavidin. After that we washed five more times with washing buffer (250 µL), A and B substrate solution (50 µL) were used for color development, plate was incubated for 15 min at 37 °C in dark environment. The detection limit for GITR, OX40L, PD-1 protein was given by the manufacturer as respectively as 1.5–24 pg/mL; 1 pg/mL–16 pg/mL; 3.75 ng/L and 60 ng/L. The sensitivity of the kit in three protein levels was 0.1 pg/mL. The optical density was read with a wavelength at 450 nm with a plate reader (Thermo Multiskan Go) after addition of 50 µL stop solution.

Statistical analysis

Statistical analysis of our study was performed by using SPSS 20 package program, ELISA values of sGITR, sOX40L and sPD-1 versus histopathological parameters, which are non-parametric tests and performed by applying t-test, Mann-Whitney U and Chi-square tests. Statistical significance was set at p values that are lower than 0.05 were regarded.

Results

According to macroscopic examinations, the localization distribution of the tumor was determined as 5% (n=1) in curvature major, 20% (n=4) in curvature minor, 15% (n=3) in cardia, 10% (n=2) in incisura angularis, 5% (n=1) in canalis pylorica, 15% (n=3) in corpus gastrica and 30% (n=6) in antrum pylorica. There was no difference in sGITR, sOX40L, sPD-1 levels according to tumor localization (p>0.05).

The demographic data and the levels of sGITR, sOX40L, sPD-1 according to histopathological stages of our study groups were shown in Table 1 as pg/mL. sGITR level was found to be 48.28 ± 2.66 pg/mL in the gastric cancer group and 39.19 ± 2.01 pg/mL in the healthy control group.

The sGITR level was found to be statistically significant in the patient group about 1.23 times higher than the control group (p=0.014). Similarly, the sOX40L level was 6.13 ± 0.64 pg/mL in the patients and 8.68 ± 0.99 pg/mL in the control group, and the sOX40L level was found to be 1.41 times higher in the control group compared to the patients (p=0.033). sPD-1 level was 41.02 ± 1.84 pg/mL in the patients and 45.19 ± 3.86 pg/mL in the control group, and there was no statistical difference between the control and patient groups (p=0.490). However, there was a positive correlation between sOX40L and sPD-1 levels (p=0.007). When the tumors were categorized according to histological type, in signet-ring cell carcinoma (SRCC); sGITR, sOX40L and sPD-1 levels were determined as 51.19 ± 3.21 pg/mL; 5.93 ± 0.65 pg/mL, 41.06 ± 3.00 pg/mL, respectively. In other adenocarcinomas, these levels were determined as 40.73 ± 2.88 pg/mL, 5.82 ± 1.59 pg/mL, 41.10 ± 3.25 pg/mL, respectively. sGITR levels of signet-ring adenocarcinomas were found to be higher than other adenocarcinomas (p=0.04).

In the early tumor stage the serum levels of sGITR, sOX40L and sPD-1 levels were detected as 46.59 ± 3.20 pg/mL; 5.09 ± 0.83 pg/mL; 40.86 ± 3.27 pg/mL, respectively, and it was detected as 49.27 ± 3.85 pg/mL; 6.60 ± 0.83 pg/mL; 41.11 ± 2.33 pg/mL in the advanced tumor stage. Although sPD-1 and sGITR levels were higher in the advanced tumor stage compared to the early tumor stage, sOX40L levels were higher in the early tumor stage compared to the advanced tumor stage. However, the difference was not statistically significant (sGITR p=0.711; sOX40L p=0.180; sPD1 p=0.837). In lymph node metastasis, sGITR, sOX40L and sPD-1 levels were measured 48.64 ± 2.96 pg/mL, 6.00 ± 0.72 pg/mL, 41.46 ± 2.01 pg/mL, respectively whereas it was measured as 45.29 ± 2.30 pg/mL, 7.05 ± 0.19 pg/mL, 37.27 ± 3.97 pg/mL. Although sGITR and sPD-1 levels were higher in patients with lymph node involvement, sOX40L levels were higher in patients without lymph node involvement and the difference was not statistically significant (sGITR p=0.655; sOX40L; p=0.817; sPD-1 p=0.421). In patients with distant metastasis, GITR level was measured as 47.88 pg/mL in one patient and sOX40L could not be detected in patient group. Similarly, the PD-1 level in an only patient was 50.17 pg/mL. sGITR level was measured as 48.31 pg/mL in an only patient and 6.13 ± 0.06 pg/mL for sOX40L and 40.51 ± 1.87 pg/mL for sPD-1 levels in patients without distant metastasis. Despite the fact that sGITR and sPD-1 levels were observed to be high in the absence of distant metastasis, the comparison could not be made statistically because the data that could not be detected or detected in one patient (p value not shown).

In the presence of perineural invasion; sGITR, sOX40L and sPD-1 levels were identified as 54.04 ± 4.87 pg/mL, 5.74 ± 1.39 pg/mL, 41.96 ± 3.20 pg/mL, respectively, but in patients without perineural invasion were identified as 45.63 ± 3.01 pg/mL, 6.37 ± 0.68 pg/mL, 40.59 ± 2.33 pg/mL. In the presence of angiolymphatic invasion; sGITR, sOX40L and sPD-1 levels were found to be 51.43 ± 6.20 pg/mL, 6.03 ± 0.84 pg/mL, 45.77 ± 3.18 pg/mL, and in the absence of that; 47.16 ± 2.94 pg/mL, 6.15 ± 0.77 pg/mL, 39.32 ± 2.11 pg/mL. In patients with perineural and angiolymphatic invasion, sGITR and sPD-1 levels were found to be high, while sOX40L was found to be low. However, this difference is not statistically significant (sGITR p=0.152; sOX40L p=0.713; sPD1 p=0.701).

sGITR, sOX40L and sPD-1 levels in patients with tumor necrosis were found to be 45.35 ± 5.37 pg/mL, 5.61 ± 1.26 pg/mL, 45.47 ± 5.33 pg/mL, and in patients without tumor necrosis were 48.83 ± 3.03 pg/mL, 6.21 ± 0.72 pg/mL, 40.18 ± 1.96 pg/mL, respectively (sGITR p=0.634; sOX40L p=0.700; sPD-1 p=0.421). In well differentiated; sGITR, sOX40L, sPD-1 levels were 40.59 ± 3.25 pg/mL, 5.36 ± 1.59 pg/mL, 44.28 ± 3.55 pg/mL, respectively whereas 50.25 ± 5.78 pg/mL, 5.88 ± 1.47 pg/mL, 40.57 ± 4.20 pg/mL in poorly differentiated (sGITR p=0.400; sOX40L p=0.800; sPD-1 p=0.400). Accordingly, sGITR and sOX40L levels were low in necrotic and well

Table 1: sGITR, sOX40L, sPD-1 levels and histo/clinicopathological changes in patients.

Demographic information	**Patients n (%)**			**Controls n (%)**		
Age (year) ± (SE)	57.78 ± 3.10			49.07 ± 2.94		
Gender						
Male	12 (60%)			24 (70.6%)		
Female	8 (40%)			10 (29.4%)		
Total	20			34		
Soluble protein levels (pg/mL)	sGITR		sOX40L		sPD-1	
	Patients mean± (SE)	Controls mean± (SE)	Patients mean± (SE)	Controls mean± (SE)	Patients mean± (SE)	Controls mean ± (SE)
	48.28 ± 2.66*	39.19 ± 2.01	6.13 ± 0.64	8.68 ± 0.99**	41.02 ± 1.84	45.19 ± 3.86
Histopathology	Patients mean ± SE (n)					
Tumor state						
Early stage (T1 and T2)	46.59 ± 3.20 (7)		5.09 ± 0.83 (8)		40.86 ± 3.27 (7)	
Advanced stage (T3 and T4)	49.27 ± 3.85 (13)		6.60 ± 0.83(12)		41.11 ± 2.33(13)	
Lymph node status						
Present	48.64 ± 2.96 (18)		6.00 ± 0.72 (17)		41.46 ± 2.01 (18)	
Absent	45.29 ± 2.30 (2)		7.05 ± 0.19 (3)		37.27 ± 3.97 (2)	
Distant metastasis[a.b]						
Present	47.88[a](1)		---[a](16)		50.17[a] (1)	
Absent	48.31[a](18)		6.13 ± 0.06		40.51 ± 1.87(18)	
Perineural invasion[b]						
Present	54.04 ± 4.87 (6)		5.74 ± 1.39 (6)		41.96 ± 3.20 (6)	
Absent	45.63 ± 3.01 (13)		6.37 ± 0.68 (13)		40.59 ± 2.33 (13)	
Angiolymphatic invasion[b]						
Present	51.43 ± 6.20 (5)		6.03 ± 0.84 (5)		45.77 ± 3.18 (5)	
Absent	47.16 ± 2.94 (14)		6.15 ± 0.77 (14)		39.32 ± 2.11 (14)	
Necrosis						
Present	45.35 ± 5.37 (3)		5.61 ± 1.26 (2)		45.47 ± 5.33 (3)	
Absent	48.83 ± 3.03 (17)		6.21 ± 0.72 (18)		40.18 ± 1.96 (17)	
Tumor types						
Other adeno carcinomas	40.73 ± 2.88 (8)		5.82 ± 1.59 (9)		41.10 ± 3.25 (8)	
Signet ring cell carcinoma	51.19 ± 3.21 (12)***		5.93 ± 0.65 (11)		41.06 ± 3.00 (12)	
Differentiation[b]						
Poor	50.25 ± 5.78 (4)		5.88 ± 1.47 (4)		40.57 ± 4.20 (4)	
Intermediate and well	40.59 ± 3.25 (5)		5.36 ± 1.59 (6)		44.28 ± 3.55 (5)	

Abbreviations: *p=0.014. **p=0.033. ***p=0.04. (a) Standard Error (SE) was not calculated because the relevant levels were not detected in any patient or as determined in one patient. (b) The histopathological data of some cases could not be evaluated or reached.

differentiated tumors. Moreover, sPD-1 level was also low in non-necrotic and poorly differentiated tumors and the difference was not statistically significant.

Discussion

Gastric carcinoma is the fourth most common malignancy in worldwide and is the second leading cause of death among all malignancies. This is due to the fact that gastric cancers produce symptomatic findings in the advanced stage and epidemiological studies show that the overall survival rate varies between 10 and 30% for 5 years. Etiologic factors include alcohol use, smoking, obesity, infection of *H. Pylori* and *Epstein – Barr virus* (EBV), Aspirin and NSAID use. Additionally, the hereditary factors make the risk of gastric cancer increase by 1.5–3 times [14]. The results obtained from studies on the pathophysiology, histopathology, biochemistry and genetics of gastric cancers will help to determine therapeutic targets for prognosis and prognostic, predictive markers.

PD-1 levels were associated with early stage and high-grade tumors in patients with gastric cancer in solid tumor models [15]. sPD-1 levels, which are characterized by increased inflammation, are also found to be high in peripheral blood in patients with advanced gastric cancer [16]. In cell culture-based studies, it is thought that PD-1 can cause proliferative and non-proliferative changes due to mixed histology and massive lymphocyte infiltration [17]. In particular, the relationship between microsatellite instability, which is a critical step in this process, and high PD-1 level is remarkable [18].

Histopathological and serum biomarker studies in gastric cancer suggest that sPD-1 expression is important for poor prognosis, low survival rate and as a prognostic factor [19]. In contrast, in the study of Shen et al., only in patients with *H. Pylori* (+) gastric cancer had a positive correlation in terms of PD-1 expression that compared to the control group. Moreover, there was no correlation in PD-1 levels between early and advanced neoplasia [15]. According to the results of our study, PD-1 levels did not show any changes in gastric cancer patients without *H. Pylori* infection compared to healthy controls ($p>0.05$). Contrary to other studies, the association of PD-1 level only in the *H. Pylori* (+) group raises the question of whether PD-1 may play a major role in the pathogenesis of gastric cancer or not. Although we conducted our study with a small sample, we aimed to provide homogeneity by forming only the patients without *H. Pylori* infection. In another study supporting Shen and our study, *H. Pylori* infection model was developed in gastric cell lines obtained from multipotent stem cells and thus PD-1 expression was observed high in the infected model compared to non-infected model [20].

Although there are different solid cancer researches in the literature of OX40, the studies of OX40L are very limited. OX40 was found to be high in patients with gastric cancer and it was reported to increase in stage 3 level [21]. Similarly, the role of Tumor-draining lymph nodes (TDLN) has been shown in modeling studies [22]. In the light of this information, it can be stated that OX40 plays a more active role in the advanced stages of carcinogenesis. Although there are not enough studies in gastric cancers, the change in gastrointestinal cancer groups such as high intratumoral OX40 level [23] in patients with liver metastasis, correlation of high OX40 level in bile duct cancer with low survival rate [24] and high expression of OX40 gene in colorectal cancer [25] were noteworthy. Similarly, OX40L level that is detected in colon cancer and murine tumor models has been shown to affect T cell activation, TDLN and tumor remission processes in the literature [26]. In our data, OX40L levels were statistically higher in patients with gastric cancer compared to healthy controls ($p = 0.033$). Although OX40L levels were not statistically different according to histopathological stages, it was observed that OX40L levels were higher in patients in early tumor stage without lymphatic involvement, distance metastasis and angio/perineural invasion. Despite the relationship between high OX40 level and advanced tumor stage in different cancers in the literature, the results of our study on OX40L level suggest that it may be higher in the early stages of carcinogenesis.

Proteins and m-RNAs that belong to GITR appear to be expressed in tumor tissue in different cancer types. Functional studies have shown that sGITR and sGITR agonists may play a role in tumor cytotoxicity, tumor immunity or even systemic immunity [27]. Particularly, high sGITR level was found to be average 1.23 times higher in gastric cancer in our study ($p=0.014$) as it was detected in esophageal cancers [28]. At the same time, sGITR levels were found to be average 1.25 times higher in stony signet-ring cell carcinomas compared to adenocarcinomas ($p=0.04$). The level of histopathological difference in sGITR levels is defined for the first time in the literature. Signet-ring cell carcinoma is usually observed in patients under 60 years of age and is less common than other types of adenocarcinoma. In contrast, Signet-ring cell carcinoma, which has an aggressive prognosis, is usually detected macroscopically in the T3 stage and has a higher risk of lymph node involvement than adenomas. In addition, although adenocarcinoma metastasis is mostly directed to other organs, intraperitoneal

metastasis is remarkable in patients with Signet-ring cell type [29]. The difference in sGITR level can be used in gastric and signet-ring carcinomas for early and differential diagnosis. Of course, there is a need for validation at the serum level difference and it is one of our next targets as well.

Another finding of our study was positive correlation between sOX40L and sPD-1 levels (p=0.007). Functional and clinical studies on the synergistic effects of sOX40 and sPD-1 have been shown to be positively correlated with active T-cell and follicular T-cell stimulating mechanisms [6, 7], and also the correlation between sOX40L and sPD-1 in gastric cancer cases was detected. Another functional study conducted by Li et al., has demonstrated that the inactivation of sOX40/sOX40L in liver tissue activated the PD-1/PD-L1 pathway [30]. Although similar results were obtained in murine tumor models [31], the positive correlation between sOX40L and sPD-1 levels in gastric cancer in our study suggests that both pathways may be regulated differently in the pathogenesis of gastric cancer.

Consequently, our results suggested that PD-1/PD-L1 and TNF/TNFR family members, which are very popular in the diagnosis, treatment and prognosis of different types of cancer have a role in the pathogenesis of gastric cancer and are important in histopathological carcinogenesis. Therefore, the data obtained in our study caBn shed light on the studies on cell death and immune response in gastrointestinal cancers.

Research funding: The present study was supported by a grant from the Scientific Research Projects Coordination Unit of Istanbul University (Project No: TYO-2019-33675).

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: The study design, protocols and informed consent was approved by both the Ethical Committee (Decree No. 2011-KAEK-50).

References

1. Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S. Stimulation of CD25(+) CD4(+) regulatory T cells through GITR breaks immunological self-tolerance. Nat Immunol 2002;3:135–42.
2. Zhan Y, Gerondakis S, Coghill E, Bourges D, Xu Y, Brady JL, et al. Glucocorticoid-induced TNF receptor expression by T cells is reciprocally regulated by NF-kappaB and NFAT. J Immunol 2008;181:5405–13.
3. Clouthier DL, Watts TH. Cell-specific and context-dependent effects of GITR in cancer, autoimmunity, and infection. Cytokine Growth Factor Rev 2014;25:91–106.
4. Ronchetti S, Nocentini G, Petrillo MG, Riccardi C. CD8+ T cells: GITR matters. Sci World J 2012;2012:308265.
5. W van Olffen R, Koning N, Van Gisbergen K, Wensveen F, Hoek M, Boon L, et al. GITR triggering induces expansion of both effector and regulatory CD4+ T cells in vivo. J Immunol 2009;182: 7490–500.
6. Webb GJ, Hirschfield GM, Lane PJ. OX40, OX40L and autoimmunity: a comprehensive review. Clin Rev Allergy Immunol 2016;50:312–32.
7. Rogers PR, Song J, Gramaglia I, Killeen N, Croft M. OX40 promotes Bcl-xL and Bcl-2 expression and is essential for long-term survival of CD4 T cells. Immunity 2001;15:445–55.
8. Zang X, Allison JP. The B7 family and cancer therapy: costimulation and coinhibition. Clin Canc Res 2007;13:5271–9.
9. Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. Immunity 2007;27:111–22.
10. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 2000;192:1027–34.
11. Agata Y, Kawasaki A, Nishimura H, Ishida Y, Tsubat T, Yagita H, et al. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. Int Immunol 1996;8:765–72.
12. Wu Y, Cao D, Qu L, Cao X, Jia Z, Zhao T, et al. PD-1 and PD-L1 co-expression predicts favorable prognosis in gastric cancer. Oncotarget 2017;8:64066–82.
13. Noh BJ, Kim JH, Eom DW. Prognostic significance of categorizing gastric carcinoma by PD-L1 expression and tumor infiltrating lymphocytes. Ann Clin Lab Sci 2018;48:695–706.
14. Sitarz R, Skierucha M, Mielko J, Offerhaus GJA, Maciejewski R, Polkowski WP. Gastric cancer: epidemiology, prevention, classification, and treatment. Canc Manag Res 2018;10:239–48.
15. Shen B, Qian A, Lao W, Li W, Chen X, Zhang B, et al. Relationship between Helicobacter pylori and expression of programmed death-1 and its ligand in gastric intraepithelial neoplasia and early-stage gastric cancer. Canc Manag Res 2019;11:3909–19.
16. Kamada T, Togashi Y, Tay C, Ha D, Sasaki A, Nakamura Y, et al. PD-1(+) regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer. Proc Natl Acad Sci U S A 2019;116: 9999–10008.
17. Wang B, Qin L, Ren M, Sun H. Effects of combination of anti-CTLA-4 and anti-PD-1 on gastric cancer cells proliferation, apoptosis and metastasis. Cell Physiol Biochem 2018;49:260–70.
18. Noh BJ, Kim JH, Eom DW. Prognostic significance of categorizing gastric carcinoma by PD-L1 expression and tumor infiltrating lymphocytes. Ann Clin Lab Sci 2018;48:695–706.
19. Shi B, Li Q, Ma X, Gao Q, Li L, Chu J. High expression of programmed cell death protein 1 on peripheral blood T-cell subsets is associated with poor prognosis in metastatic gastric cancer. Oncol Lett 2018;16:4448–54.
20. Holokai L, Chakrabarti J, Broda T, Chang J, Hawkins JA, Sundaram N, et al. Increased programmed death-ligand 1 is an early epithelial cell response to Helicobacter pylori infection. PLoS Pathog 2019;15: e1007468.
21. Martins MR, Santos RLD, Jatahy KDN, Matta MCD, Batista TP, Júnior JIC, et al. Could OX40 agonist antibody promote activation of the anti-tumor immune response in gastric cancer?. J Surg Oncol 2018;117:840–4.

22. Hebb JPO, Mosley AR, Vences-Catalán F, Rajasekaran N, Rosén A, Ellmark P, et al. Administration of low-dose combination anti-CTLA4, anti-CD137, and anti-OX40 into murine tumor or proximal to the tumor draining lymph node induces systemic tumor regression. Cancer Immunol Immunother 2018;67: 47–60.
23. Cepowicz D, Zaręba K, Gryko M, Stasiak-Bermuta A, Kędra B. Determination of the activity of CD134 (OX-40) and CD137 (4-1BB) adhesive molecules by means of flow cytometry in patients with colorectal cancer metastases to the liver. Pol Przegl Chir 2011;83: 424–9.
24. Chellappa S, Hugenschmidt H, Hagness M, Subramani S, Melum E, Line PD, et al. CD8+ T cells that coexpress RORγt and T-bet are functionally impaired and expand in patients with distal bile duct cancer. J Immunol 2017;198:1729–39.
25. Weixler B, Cremonesi E, Sorge R, Muraro MG, Delko T, Nebiker CA, et al. OX40 expression enhances the prognostic significance of CD8 positive lymphocyte infiltration in colorectal cancer. Oncotarget 2015;6:37588–99.
26. Haabeth OAW, Blake TR, McKinlay CJ, Tveita AA, Sallets A, Waymouth RM, et al. Local delivery of Ox40l, Cd80, and Cd86 mRNA kindles global anticancer immunity. Canc Res 2019;79:1624–34.
27. Narumi K, Miyakawa R, Shibasaki C, Henmi M, Mizoguchi Y, Ueda R, et al. Local administration of GITR agonistic antibody induces a stronger antitumor immunity than systemic delivery. Sci Rep 2019;9:5562.
28. von Rahden BH, Kircher S, Kafka M, Stuermer L, Reiber C, Gattenlöhner S, et al. Glucocorticoid-induced TNFR family-related receptor (GITR)-expression in tumor infiltrating leucocytes (TILs) is associated with the pathogenesis of esophageal adenocarcinomas with and without Barrett's mucosa. Canc Biomarkers 2010;7:285–94.
29. Machlowska J, Pucułek M, Sitarz M, Terlecki P, Maciejewski R, Sitarz R. State of the art for gastric signet ring cell carcinoma: from classification, prognosis, and genomic characteristics to specified treatments. Canc Manag Res 2019;11:2151–61.
30. Li T, Ma R, Zhu J, Wang F, Huang L, Leng X. Blockade of the OX40/OX40L pathway and induction of PD-L1 synergistically protects mouse islet allografts from rejection. Chin Med J 2014;127: 2686–92.
31. Fromm G, de Silva S, Johannes K, Patel A, Hornblower JC, Schreiber TH. Agonist redirected checkpoint, PD1-Fc-OX40L, for cancer Immunotherapy. J Immunother Cancer 2018;6:149.