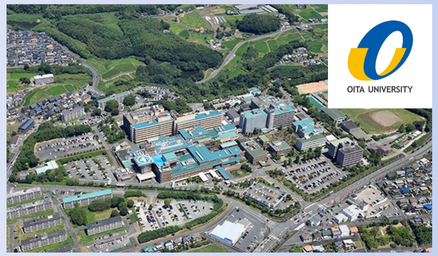

The lymphocyte activation gene-3 (LAG-3) protein expression in tumor-infiltrating lymphocytes is associated with a poor prognosis of ovarian clear cell carcinoma

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BACKGROUND

Epithelial ovarian cancer is classified into several histological types, but individual treatment for them has not been established. Ovarian clear cell carcinoma (OCCC) is resistant to standard therapy for epithelial ovarian cancer and requires a new effective therapy. Recently, in OCCC, co-inhibition with two immune checkpoints; programmed death ligand-1 (PD-L1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) are potential to more effective strategy for the patient with OCCC than other histological types[1)], including several problems; no evasion of adverse events and lack of useful biomarker for prediction of the efficacy. The solution of these problems will be the research a new immune checkpoint molecule. Lymphocyte activation gene 3 (LAG-3) is the third immune checkpoint followed by PD-1 and CTLA-4. Co-inhibition of LAG-3 and PD-1 was suggested as the new effective therapy and might have fewer adverse events than co-inhibition of CTLA-4 and PD-1 in a randomized phase III of melanoma[2)]. In a mouse model of solid tumors, co-inhibition of LAG-3 and PD-1 enhanced antitumor activity compared with single-inhibition of immune checkpoint[3)]. It was reported that tumor-infiltrating lymphocytes (TILs) expressing LAG-3 in ovarian cancer is correlation with decreased effector function of PD-1$^+$ CD8$^+$ T cells *in vitro*[4)]. However, it have been no studies about the correlation of LAG-3 expression and patient prognosis in OCCC. Our research is the first report about the correlation between LAG-3 positive TILs and their clinical features.

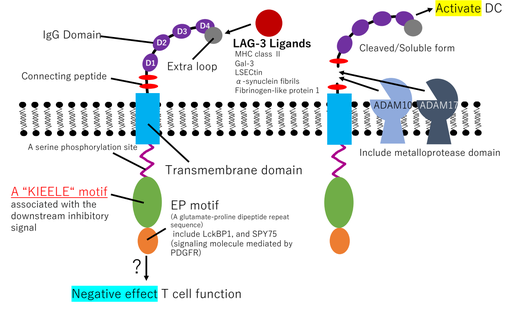

Fig 1. Scheme of Lymphocyte activation gene 3 (LAG-3): LAG-3 is expressed on the surface of immune cells, including T cells, and have correlation with T cell exhaustion, which is negative regulation of T cells resulted by persistent inflammation and over-expression of multiple immune checkpoints. LAG-3 has negative regulation of T cell when it is on cell surface. On the other hand, LAG-3 activate dendritic cell when it is cleaved form. However, a detailed mechanism of these immune regulation is unclear.

MATERIALS and METHODS

171 patients with OCCC was recruited. The clinicopathological features; age, recurrence/progression-free survival (PFS), death/overall survival (OS), FIGO stage, surgical status (complete resection or incomplete resection), and treatment methods were reviewed (Table 1). We used a tissue microarray for analyzing immunohistochemical expression of LAG-3 and defined more than 20% of TILs stained brown as positive (Fig 2). We analyzed the correlation between the clinicopathological features and LAG-3 expression.

Table 1. Characteristics of patients with OCCC.

Characteristic	N (%)
Age	
Median (range)	55.3 (32–80)
<55	79 (46.2)
≥55	92 (54.1)
FIGO stage	
I	111 (64.9)
II	25 (14.6)
III	31 (18.1)
IV	4 (2.3)
Surgical procedures	
TAH + BSO + OM	142 (83.0)
BSO + OM	6 (3.5)
USO + OM	8 (4.7)
OM	15 (8.8)
Surgical status	
Complete resection	146 (85.4)
Incomplete resection	25 (14.6)
Adjuvant chemotherapy	
Yes	129 (75.4)
Paclitaxel + Carboplatin	77
Docetaxel + Carboplatin	35
Irinotecan + Cisplatin	12
Gemcitabine + Carboplatin	1
Not available	4
No	42 (24.6)
Recurrence	
Yes	44 (25.7)
No	127 (74.3)

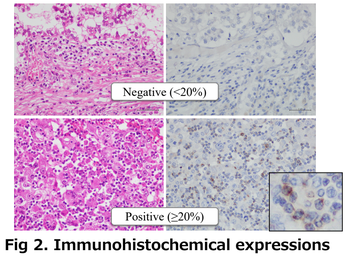

Fig 2. Immunohistochemical expressions

RESULTS

Table 2. The correlation between patient characteristics and LAG-3 expression with OCCC.

Characteristic		LAG-3 Positive	Negative	*P*-value
ALL		48	123	
Age (Median = 55.3)	<55	67	56	0.613
	≥55	24	24	
FIGO stage	**I+II**	**33**	**103**	**0.036**
	III+IV	**15**	**20**	
Residual tumor	Yes	10	15	0.156
	No	38	108	
Recurrence	**Yes**	**19**	**25**	**0.012**
	No	**29**	**98**	
Death	Yes	13	19	0.086
	No	35	104	

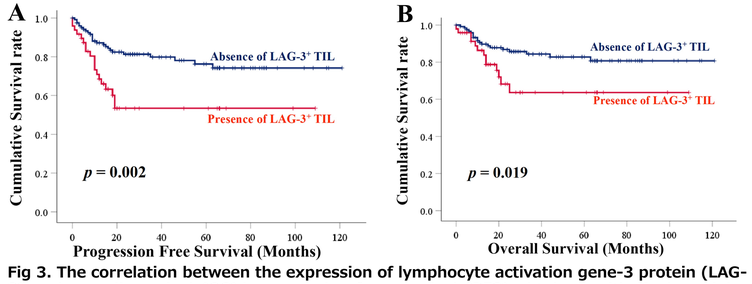

Fig 3. The correlation between the expression of lymphocyte activation gene-3 protein (LAG-3) and overall survival (OS)/ progression-free survival (PFS) obtained with Kaplan–Meier statistical analysis and log-rank test in patients with ovarian clear cell carcinoma (OCCC): (A) PFS and LAG-3, (B) OS and LAG-3.

Table 3. Univariable and multivariable analysis for PFS in patients with OCCC.

	Univariate analysis			Multivariate analysis		
Characteristics	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age	0.88	0.49–1.60	0.679			
FIGO stage	**5.37**	**2.95–9.77**	**<0.001**			
LAG-3	**2.53**	**1.39–4.61**	**0.002**	**1.86**	**1.00-3.44**	**0.049**
Residual tumor	**11.11**	**5.95–20.83**	**<0.001**	**9.71**	**5.13-18.52**	**<0.001**

CONCLUSIONS

This study is the first to investigate that LAG-3 is correlated with high FIGO stages and recurrence and LAG-3 is a poor prognostic factor in OCCC. These may suggest that LAG-3 expression reflect decreasing anti-tumor activity resulted by persistent inflammation in OCCC.

Conversely, several studies have demonstrated that LAG-3 expression is correlated with better prognosis. This may be relationship with activation dendritic cell by soluble LAG-3 separated from cell surface.

It was reported that PD-1$^+$/LAG-3$^+$/CD8$^+$ TILs from ovarian cancer patients regained antitumor activity after co-inhibition of LAG-3 and PD-1 *in vitro*[4)]. Immune checkpoint inhibitor via LAG-3 is a potential therapeutic target and predictive biomarker for LAG-3 positive ovarian clear cell carcinoma with poor prognosis.

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The authors declare no competing interests.
This report is currently under submission to the journal of ovarian research.