Expression of lymphocyte-activating gene 3 and T-cell immunoreceptor with immunoglobulin and ITIM domains in cutaneous melanoma and their correlation with programmed cell death 1 expression in tumor-infiltrating lymphocytes

Woo Jin Lee, MD, PhD, Ye Jin Lee, MD, Myoung Eun Choi, MD, Kyung A. Yun, MSc, Chong Hyun Won, MD, PhD, Mi Woo Lee, MD, PhD, Jee Ho Choi, MD, PhD, and Sung Eun Chang, MD, PhD
Seoul, Korea

Background: Lymphocyte-activating gene 3 (LAG-3) and T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibition motif (TIGIT) domains are emerging checkpoint proteins.

Objective: We evaluated LAG-3 and TIGIT protein expression patterns, correlated these patterns with programmed cell death 1 (PD-1) protein expression, and determined their effects on clinicopathologic characteristics and biologic responses in melanoma.

Methods: Diagnostic tissue from 124 patients with melanoma were evaluated for LAG-3, TIGIT, and PD-1 expression by immunohistochemistry. Clinicopathologic features and survival were analyzed according to the expression of LAG-3, TIGIT, and PD-1.

Results: LAG-3 and TIGIT expression on tumor-infiltrating lymphocytes were significantly correlated with that of PD-1 and was also significantly associated with negative prognostic factors: deeper Breslow thickness, lymph node involvement, and advanced stage of disease. However, PD-1 expression was not associated with clinicopathologic variables of prognostic significance. High expression of either LAG-3 or TIGIT was associated with worse survival. Subgroup analysis on the basis of Breslow thickness showed that both LAG-3 and TIGIT have prognostic significance regardless of tumor thickness. High expression of PD-1 was not predictive of survival.

Limitations: Retrospective study in a single institution and possibility of type 1 error.

Conclusion: Expression of LAG-3 and TIGIT represents an independent unfavorable prognostic factor in cutaneous melanoma. (J Am Acad Dermatol 2019;81:219-27.)

Key words: LAG-3; melanoma; PD-1; survival; TIGIT; tumor-infiltrating lymphocytes.

In various cancer types, there has been an increasing focus on the tumor microenvironment as a target for anticancer treatment, the strategy being to reverse tumor immune escape through the suppression of immune checkpoints.[1] Lymphocyte-activating gene 3 (LAG-3) and T-cell

From the Department of Dermatology, Asan Medical Center, University of Ulsan College of Medicine, Seoul.
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Correspondence to: Woo Jin Lee, MD, PhD or Sung Eun Chang, MD, PhD, Department of Dermatology, Asan Medical Center, University of Ulsan College of Medicine, 388-1 Pungnap-dong Songpa-gu, Seoul 138-736, Korea. E-mail: Woo Jin Lee (uucm79@hanmail.net) or Sung Eun Chang (csesnumd@gmail.com).
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immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains (TIGIT) comprise the next generation of co-inhibitory receptors to be used in anticancer therapy. LAG-3 is an important checkpoint molecule that might have a synergistic effect with programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1).[2-4] LAG-3 is expressed on natural killer cells, B cells, tumor-infiltrating lymphocytes (TILs), and dendritic cells.[5] LAG-3 binds to major histocompatibility complex II on TILs and inhibits T-cell proliferation, activation, and homeostasis.[5] Although LAG-3 has been evaluated in many malignancies, including hematologic and solid organ tumors,[6] its expression in melanoma has not been evaluated.

CAPSULE SUMMARY

- Lymphocyte-activating gene 3 (LAG-3) and T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains (TIGIT) are emerging checkpoint proteins. LAG-3 and TIGIT expression have prognostic value and are correlated with programmed cell death 1 expression in melanoma.
- Combined checkpoint blockade of LAG-3 and TIGIT might be of therapeutic value in melanoma.

TIGIT is a CD28 family–like receptor expressed by natural killer cells, regulatory T cells, and exhausted $CD8^+$ T cells.[7] TIGIT has 2 agonists, CD155 and CD112, and is expressed by immune cells and tumor cells.[8] It exerts suppressive effects on immune cells by increasing the release of immunoregulatory cytokines, such as interleukin (IL) 10.[8] Blocking TIGIT enhances $CD8^+$ T-cell effector functions in tumor-bearing mice.[9] TIGIT can be highly expressed in TILs, along with PD-1 and LAG-3.[10] TIGIT expression in melanoma tumor tissue has not been evaluated.

Considering that combination PD-1 and LAG-3 or TIGIT antibodies have emerged as potential anticancer therapies and in clinical trials, an evaluation of the relationship between PD-1 and LAG-3 or TIGIT expression is needed. The prognostic significance of LAG-3 and TIGIT expression in cutaneous melanoma and its correlation with PD-1 expression has not been evaluated. In this study, we investigated LAG-3 and TIGIT expression in melanoma tissue and their correlation with PD-1 expression by immunohistochemistry. We also analyzed the clinicopathologic features of LAG-3 and TIGIT expression and their biologic behaviors.

METHODS

The database of Asan Medical Center was searched for cases of cutaneous melanoma that were confirmed by skin biopsy during January 1998-December 2016.

Evaluation of LAG-3, TIGIT, and PD-1 expression, and TILs

Paraffin-embedded sections were immunostained with anti-PD-1 (clone NAT105, 1:100; Abcam, Cambridge, MA), anti-LAG-3 (clone 11E3, 1:2000; Abcam), and anti-TIGIT (clone TG1, 1:100; Biomatik, Wilmington, DE). We calculated the number of lymphocytes in the microscopic field of vision in hematoxylin-eosin–stained sections. TILs were graded by using a semiquantitative scheme that was based on their density in tumor tissue, with grades 0-3 (zero meaning absence of lymphocytes within the tissue, 1 meaning the presence of lymphocytes occupying <25% of the tissue, 2 meaning presence of lymphocytes occupying 25%-50% of the tissue, and 3 meaning presence of lymphocytes occupying >50% of the tissue). Scores of 2 or 3 were considered to represent a high density of TILs. The percentage of TILs showing positive cytoplasmic membrane staining for LAG-3, TIGIT, and PD-1 was determined. Intratumoral and peritumoral expression of LAG-3 and TIGIT was evaluated. Peritumoral staining was evaluated within the peritumoral immune cells that were in direct contact with the tumor cells at the boundary of the tumor mass. The intensity of staining was determined on a scale of 0-3, with zero indicating <5%, 1 indicating 5%-20%, 2 indicating >20%-50%, and 3 indicating >50% of TILs). Cases with a score ≥1 were considered positive. Scores of 2 and 3 were considered to represent high expression of LAG-3, TIGIT, and PD-1. All samples were evaluated independently by 2 investigators (Dr W.J. Lee, Dr Y.J. Lee). Scoring agreement between the 2 investigators was measured with the Kappa coefficient. For cases in which different scores were provided by the 2 investigators, re-evaluation was performed by using a double-headed microscope.

Immunofluorescent staining

Immunofluorescent double staining was carried out with formalin-fixed, paraffin-embedded sections after heat antigen retrieval following standard protocols. The following primary antibodies were used: CD3 antibody (clone PS1, 1:50; Abcam), LAG-3 (clone 17B4, 1:100; Abcam), TIGIT antibody (1:10, Bioscience, San Diego, CA), and PD-1 antibody

Abbreviations used:

AJCC: American Joint Committee on Cancer
CI: confidence interval
HR: hazard ratio
LAG-3: lymphocyte-activating gene 3
OS: overall survival
PD-1: programmed cell death 1
PD-L1: programmed death-ligand 1
PFS: progression-free survival
TIGIT: T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains
TILs: tumor-infiltrating lymphocytes

(AF1021, 1:50, R&D systems, Minneapolis, MN). As secondary antibody, we used a FITC-conjugated donkey—anti-sheep IgG (1:100; Serotec, Düsseldorf, Germany) to detect the CD3 antibody and Alexa Fluor 568 (1:500; Invitrogen, Waltham, MA) to detect LAG-3, TIGIT, and PD-1 antibodies.

Statistical analysis

All analyses were performed by using statistical software package SPSS, version 18.0 (SPSS Inc, Chicago, IL). A P value $<.05$ was considered statistically significant. Survival was plotted by using the Kaplan—Meier method, and comparisons were made between subgroups with high and low expression of immune checkpoints by using the log-rank test. Prognostic factors independently associated with overall survival (OS) and progression-free survival (PFS) at the time of diagnosis were identified by multivariate analysis using Cox proportional hazards regression modelling. Clinicopathologic comparisons between subgroups with high or low expression of immune checkpoints were performed by using chi-squared tests for categorical variables and t tests for continuous variables. Pearson's correlation coefficient was used to evaluate associations for continuous variables in LAG-3, TIGIT, and PD-1 expression.

RESULTS

A total of 124 cases of cutaneous melanoma were included in the study. The demographic data and clinical features of the patients are summarized in Table I.

Expression of LAG-3, TIGIT, and PD-1 and their association with clinicopathologic features

LAG-3 was expressed in 77 (62.1%) and TIGIT in 75 (60.5%) of the stained tissue specimens from 124 patients. PD-1 was expressed in 96 (77.4%) of these tumor tissues. The 2 investigators agreed on LAG-3 expression in 109 of 124 (88%, k = 0.653) tumors, TIGIT expression in 116 (94%, k = 0.573) tumors, and PD-1 expression in 110 (89%, k = 0.639) tumors. Clinicopathologic variables were stratified depending on the expression of these proteins in tumor tissues to assess for associations (Table II).

Table I. Clinicopathologic characteristics of 124 patients with cutaneous melanoma

Clinical feature at diagnosis	No. patients, N = 124, n (%)
Sex	
Male	68 (54.8)
Female	56 (45.2)
Age, years	
Range	25-89
Mean	61.8
Extracutaneous involvement at diagnosis	
Lymph node involvement	28 (22.6)
Visceral involvement	12 (9.68)
AJCC stage at diagnosis	
I and II	91 (73.4)
III and IV	33 (26.6)
Histopathologic features at diagnosis	
Breslow thickness, mm	
≤1 T1	21 (16.9)
>1 to ≤2 T2	47 (37.9)
>2 to ≤4, T3	32 (25.8)
>4, T4	24 (19.4)
Ulceration	
Yes	36 (29.0)
No	88 (70.1)
Vertical growth phase	
Yes	49 (39.5)
No	75 (60.5)
Lymphovascular invasion	11 (8.87)
Mitosis, mean in 10 HPFs	18.9

AJCC, American Joint Committee on Cancer; *HPF*, high-power field.

Of the 124 patients, 55 (44.4%) showed high expression of LAG-3, 48 (38.7%) high peritumoral LAG-3 expression, and 34 (27.4%) high intratumoral LAG-3 expression (Fig 1; Table II). There were significant correlations between the high expression of LAG-3 and pathologic findings, such as a deeper Breslow thickness ($P < .001$) and more vertical growth ($P < .001$; Table II). Mitosis was significantly more frequent in patients with high LAG-3 expression ($P = .027$). High LAG-3 expression was also associated with a higher frequency of lymph node involvement ($P < .001$) and a higher frequency of advanced American Joint Committee on Cancer (AJCC) stage ($P = .001$; Table II).

High TIGIT expression was found in 52 of 124 (41.9%) patients. The frequency of high peritumoral TIGIT expression (46/124, 37.1%, Fig 1) was higher than that of high intratumoral TIGIT expression

Table II. Clinicohistopathologic characteristics of 124 cutaneous melanomas according to LAG-3, TIGIT, and PD-1 expression

Characteristic	LAG-3 expression			TIGIT expression			PD-1 expression		
	Score 0 or 1, n = 69	Score 2 or 3, n = 55	*P* value	Score 0 or 1, n = 72	Score 2 or 3, n = 52	*P* value	Score 0 or 1, n = 60	Score 2 or 3, n = 64	*P* value
Breslow thickness, mm			<.001*			.001*			.744
≤1, T1, n = 21	15	6		15	6		9	12	
>1 to ≤ 2, T2, n = 47	34	13		34	13		23	24	
>2 to ≤ 4, T3, n = 32	12	20		14	18		17	15	
>4, T4, n = 24	8	16		9	15		11	13	
Ulceration			.418			.244			.868
Yes, n = 36	18	18		18	18		17	19	
No, n = 88	51	37		54	34		43	45	
Vertical growth phase			<.001*			<.001*			.915
Yes, n = 49	17	32		19	30		24	25	
No, n = 75	52	23		53	22		36	39	
Lymphovascular invasion			.476			.804			.838
Yes, n = 11	5	6		6	5		5	6	
No, n = 113	64	49		66	47		55	58	
Mitosis, mean in 10 HPFs	11.2	24.6	.027*	13.7	22.5	.041*	16.7	19.4	.187
Sex			.302			.358			.263
Male, n = 68	35	33		42	26		36	32	
Female, n = 56	34	22		30	26		24	32	
Age, y									
<60, n = 66	38	28		37	29		32	34	
≥60, n = 58	31	27		35	23		28	30	
Extracutaneous involvement			.644			.630			.981
Lymph node, n = 28	7	21		10	18		14	14	
Viscera, n = 12	3	9		4	8		4	8	
AJCC stage			<.001*			.006*			.846
I and II, n = 91	59	32	.033*	58	33	.121	46	45	.322
III and IV, n = 33	10	23	.001*	14	19	.034*	14	19	.424

AJCC, American Joint Committee on Cancer; *HPF*, high-power field; *LAG-3*, lymphocyte-activating gene 3; *PD-1*, programmed cell death 1; *TIGIT*, T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains.
*Statistically significant.

(30/124, 24.2%, Fig 1). High TIGIT expression was associated with pathologic variables, such as deeper Breslow thickness (P = .001) and more vertical growth (P < .001; Table II). Mitotic counts were significantly higher in patients with high TIGIT expression (P = .041). High expression of TIGIT was associated with a higher frequency of lymph node involvement (P = .006) and advanced AJCC stage (P = .034) (Table II).

Of 124 patients, 64 (51.6%) showed PD-1 expression with scores of 2 or 3. In total, 55 patients (44.3%) showed high peritumoral PD-1 expression, and 44 (35.5%) showed high intratumoral PD-1 expression (Fig 1). There were no significant differences in the measured tumor depth with respect to the density of PD-1 positivity (Table II). Clinical variables, such as advanced AJCC stage (P = .424), visceral involvement (P = .322), and lymph node involvement (P = .846), were not associated with high PD-1 expression.

Correlation of LAG-3, TIGIT, and PD-1 expression

Expression of LAG-3 (P = .012), TIGIT (P < .001), and PD-1 (P < .001) were correlated with the density of TILs. Of 64 cases with high PD-1 expression, 35 (54.7%) also had high LAG-3 expression, and there was a significant association between PD-1 and LAG-3 expression (P = .017). Expression of TIGIT was also associated with PD-1 (P < .001). When expression level was evaluated as a continuous variable, LAG-3 (rho 0.705, P < .001) and TIGIT (rho 0.82, P < .001) expression were correlated with PD-1 expression. Double immunofluorescent stainings showed that PD-1−positive cells also expressed LAG-3 and TIGIT (Fig 2).

Prognostic significance of LAG-3, TIGIT, and PD-1 expression

The median follow-up was 71 (range 22-137) months. When all patients were combined into a

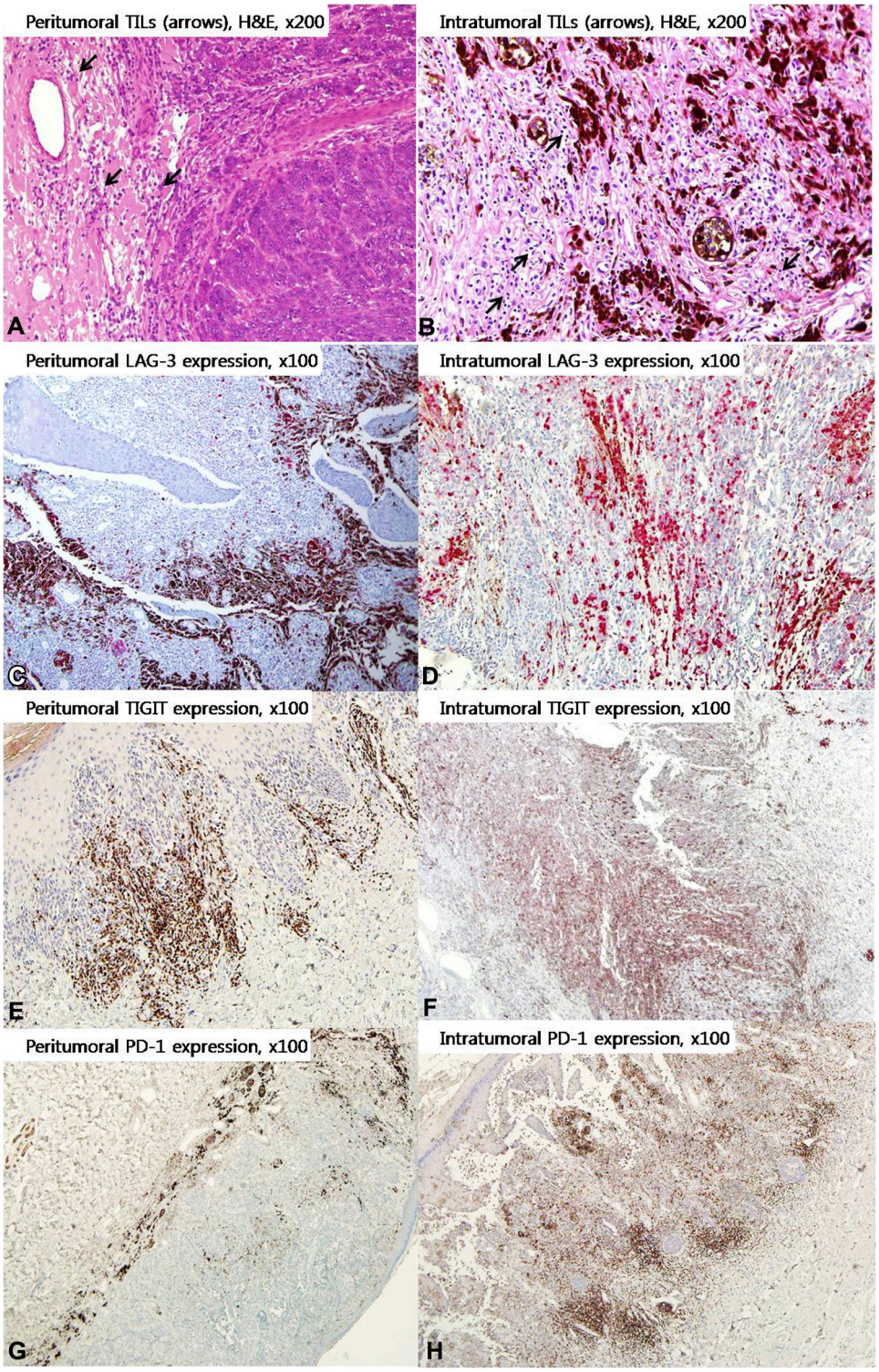

Fig 1. Histopathologic features and expression of LAG-3, TIGIT, and PD-1 in cutaneous melanoma. **A** and **B**, TILs were found in peritumoral or intratumoral areas (*arrows*). TILs expressed LAG-3 (**C** and **D**), TIGIT (**E** and **F**), and PD-1 (**G** and **H**) in peritumoral or intratumoral areas. *LAG-3*, Lymphocyte-activating gene 3; *PD-1*, programmed death cell 1; *TIGIT*, T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains; *TILs*, tumor-infiltrating lymphocytes. (**A** and **B**, Hematoxylin-eosin stain: ×200; **C-H**, immunostain: ×100.)

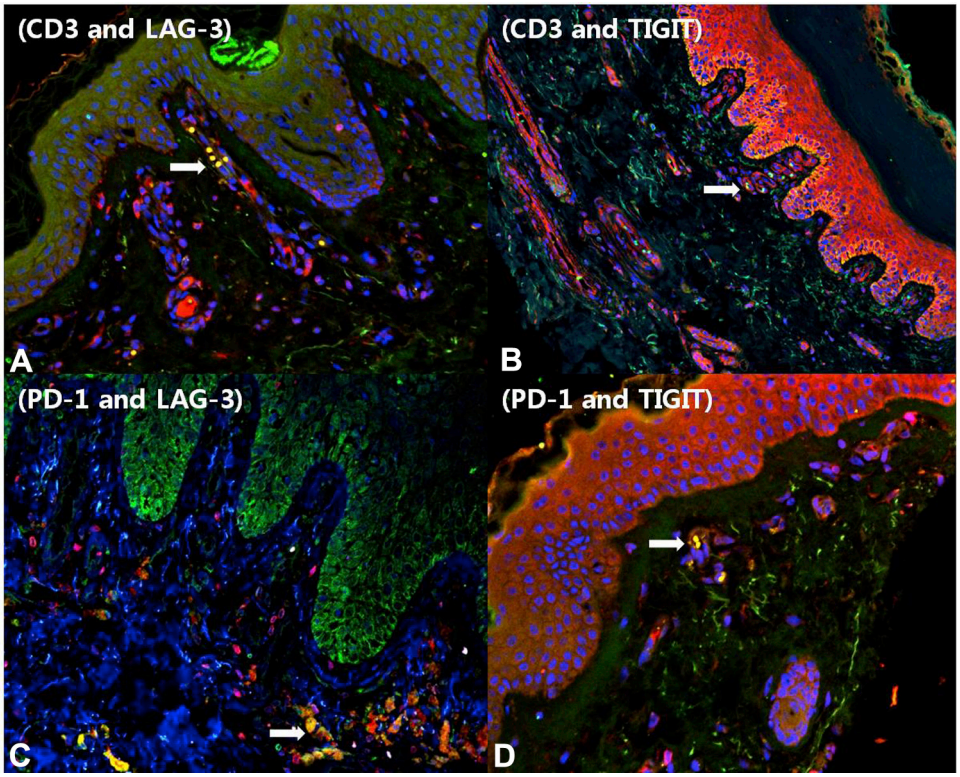

Fig 2. Coexpression of proteins in tumor tissue. Double immunofluorescence staining of CD3 (*red*) and LAG-3 (*green*) (**A**), CD3 (*red*) and TIGIT (*green*) (**B**), PD-1 (*red*) and LAG-3 (*green*) (**C**), and PD-1 (*red*) and TIGIT (*green*) (**D**) in melanoma tissue. Double-positive cells are yellow (*arrows*). *LAG-3*, Lymphocyte-activating gene 3; *PD-1*, programmed death cell 1; *TIGIT*, T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains.

single cohort, the 5-year OS rate was 48%, and the median OS period was 62.0 (95% confidence interval [CI] 49.26-83.47) months.

OS was significantly shorter in patients with high expression (median OS 59.0 months, 95% CI 40.80-77.19 months) than patients with low expression of LAG-3 (median OS 74.0 months, 95% CI data not found; P = .017, Fig 3). PFS was also significantly better in patients with low expression of LAG-3 (59.0 months) than those with high expression of LAG-3 (36.0 months, P = .035). The prognostic value of peritumoral LAG-3 expression for OS (P = .019) was more significant than that of intratumoral LAG-3 expression for OS (P = .046). Patients with high TIGIT expression showed inferior OS (median 59.0 months, 95% CI 43.39-74.60 months) compared with that of patients with low TIGIT expression (median OS 74.0 months, 95% CI 62.67-85.32 months, P = .030; Fig 3). PFS was also significantly worse in patients with high expression than patients with low expression of TIGIT (59.0 months vs 32.0 months, P = .010). Peritumoral TIGIT expression was predictive of poor OS (P = .032), but intratumoral TIGIT expression was not significantly associated with poor OS (P = .060). Subgroup analysis by Breslow thickness showed that both LAG-3 (Fig 3) and TIGIT (OS, P = .031; PFS, P = .041) had prognostic significance regardless of tumor thickness.

We found no significant differences in OS (P = .639) and PFS (P = .856) on the basis of PD-1 expression. Poor OS was associated with the expression of LAG-3 in patients with high expression of PD-1 (P = .037). Expression of TIGIT also predicted worse OS in patients with high expression of PD-1 (P = .026).

Univariate analysis revealed that LAG-3 expression (hazard ratio [HR] 2.39, 95% CI 1.39-4.88, P = .033) and TIGIT expression (HR 1.98, 95% CI 1.26-5.67, P = .034) indices, deeper Breslow thickness (HR 2.19, 95% CI 1.21-5.55, P = .028), and advanced AJCC stage (HR 2.41, 95% CI 1.24-4.28, P = .023) were associated with poor OS. Multivariate analysis with these variables revealed that LAG-3 (HR 1.86, 95% CI 1.16-4.74, P = .040) and TIGIT (HR 1.45, 95% CI 1.11-5.05, P = .047) expression were independent prognostic markers for lower OS.

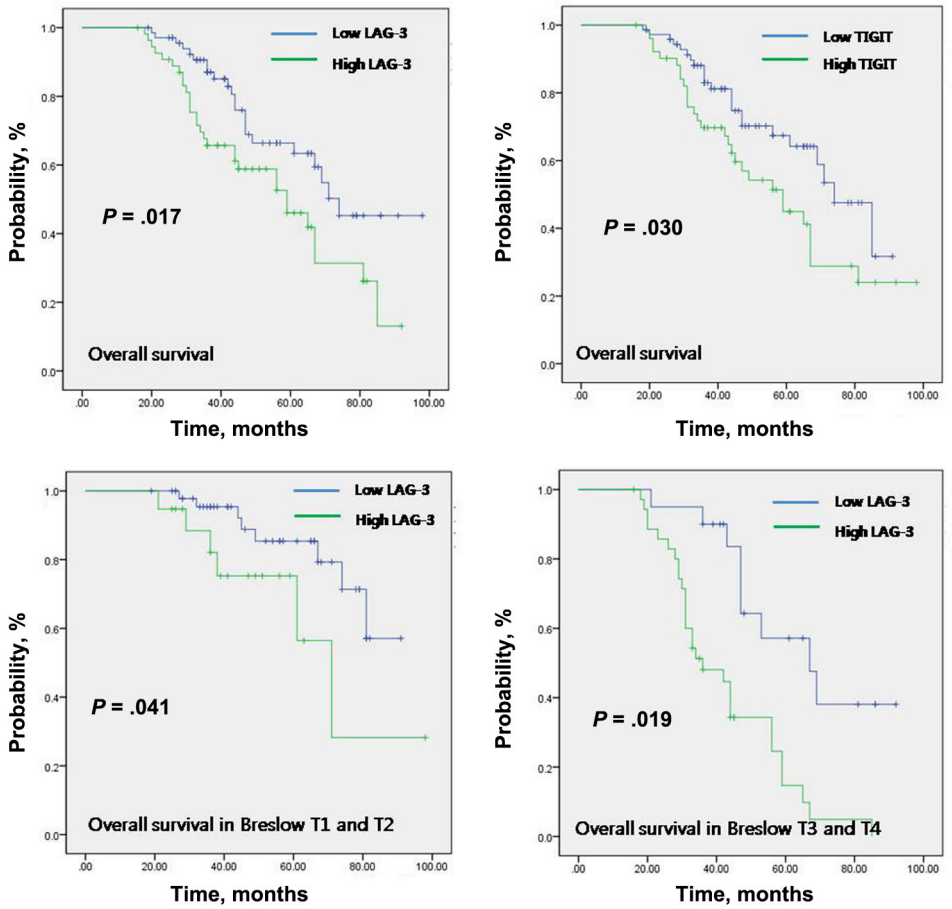

Fig 3. Comparison of overall survival according to LAG-3 and TIGIT expression. Differences in overall survival depending on LAG-3 were noted regardless of Breslow thickness. *LAG-3*, Lymphocyte-activating gene 3; *TIGIT*, T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains.

DISCUSSION

LAG-3 is generally absent on resting T cells and is upregulated on activated T cells.[5] Previous in vivo studies demonstrated that $CD4^+$ T cells exhibit increased proliferation and enhanced cytokine production when LAG-3 is blocked.[11] LAG-3 is an emerging immune checkpoint molecule and has been studied in several malignancies.[12,13] LAG-3 is coexpressed with PD-1 in breast cancer tissue and positively correlated with the presence of TILs.[12] In melanoma, LAG-3–mediated activation of plasmacytoid dendritic cells takes place in vivo at tumor sites and is in part responsible for directing an immune-suppressive environment.[14] In addition to the negative regulation of T-cell proliferation, LAG-3–major histocompatibility complex II interactions contribute to melanoma resistance by preventing apoptosis of melanoma cells through activation of mitogen-activated protein kinase and extracellular signal-regulated kinase and phosphoinositide-3 kinase and Akt pathways.[15]

TIGIT is often expressed in $CD8^+$ TILs in nonsmall cell lung and colon cancers, and TIGIT-expressing $CD8^+$ T cells often coexpress the inhibitory receptor PD-1.[9] TIGIT blockade could synergize with PD-L1 and T-cell immunoglobulin and mucin domain–containing 3 blockade to enhance T-cell functions and anti-tumor immune responses.[9] TIGIT can suppress antitumor immunity both by direct suppression of effector $CD8^+$ T cells and by indirect suppression via promotion of T-cell regulatory function.[16,17] Ex vivo experiments demonstrated that TIGIT is upregulated on serum tumor antigen–specific $CD8^+$ T cells and $CD8^+$ TILs from patients with melanoma.[18] In addition, CD155 expression is increased in melanoma cells, and the anti-melanoma cytotoxic T-cell response is inhibited via TIGIT–CD155 interactions in melanoma cells.[19]

Although the function of LAG-3 and TIGIT in tumorigenesis of melanoma cells were evaluated,[15,18,19] their expression in melanoma

tissue and prognostic significance were not investigated. In the present study, we found that LAG-3 and TIGIT were expressed in $CD3^+$ TILs, and their expression was correlated with the density of TILs. Double immunofluorescent staining of melanoma tissue showed that $CD3^+$ TILs expressed LAG-3 and TIGIT (Fig 2). PD-1–positive cells also expressed LAG-3 and TIGIT (Fig 2). PD-1 is expressed by $CD3^+$ T cells after activation[20] and has a ligand called PD-L1, which is expressed on many cell types, such as T cells, dendritic cells, and tumor cells.[20] PD-L1 expression on tumor cells can be used to predict the response to anti-PD-1 and PD-L1 immunotherapy.[21,22] Tumoral PD-L1 expression was not evaluated in this study. Because LAG-3 and TIGIT are expressed on TILs but not on tumor cells, we evaluated the relationship between PD-1 and LAG-3 or TIGIT expression on TILs. The frequency of positivity was higher for PD-1 than LAG-3 and TIGIT, and prognostic significance was noted for LAG-3 and TIGIT expression but not PD-1 expression.

In this study, LAG-3 and TIGIT were associated with clinicopathologic variables that serve as indexes of disease progression and poor survival, suggesting LAG-3 and TIGIT are prognostic biomarkers and could be targets of antitumor immunotherapy. LAG-3 and TIGIT expression was higher in the peritumoral area than in the intratumoral area. The prognostic value of peritumoral staining for LAG-3 and TIGIT was also more significant than that for intratumoral staining. We performed multivariate analysis because high LAG-3 and TIGIT expression were associated with deeper Breslow thickness and advanced AJCC stage. Multivariate analysis showed that the prognostic significance of LAG-3 and TIGIT was sustained irrespective of tumor thickness and AJCC stage.

Here, we suggest that a combination of LAG-3 or TIGIT antibodies with PD-1 antibody might have important implications for immune checkpoint therapy in cutaneous melanoma. We found that PD-1 and LAG-3 or TIGIT were concurrently expressed in ~30% of melanoma cases. Our data suggested that a key role for LAG-3 and TIGIT is in PD-1–mediated T-cell exhaustion and immunosuppressive functions. PD-1 expression was not associated with clinicopathologic variables and survival data in melanoma patients. However, high expression of LAG-3 and TIGIT predicted poor survival in PD-1–positive patients. Patients with high expression of both PD-1 and LAG-3 showed significantly deeper Breslow thickness ($P = .041$) and a higher frequency of vertical growth phase ($P = .012$) compared with patients with high expression of PD-1 but not LAG-3 (data not shown). High expression of TIGIT was associated with advanced AJCC stage in patients with high PD-1 expression ($P = .009$, data not shown). These results suggest that PD-1 expression is functionally heterogeneous and other markers are needed to define the function of PD-1 expression. PD-1 could be expressed both in exhausted and activated T cells.[23] To better define the population of exhausted T cells, other molecules associated with T-cell exhaustion were investigated, and LAG-3 expression identified truly exhausted T cells in hematologic malignancy.[24] In cutaneous melanoma, the prognostic value of LAG-3 or TIGIT expression is higher than that for PD-1 expression, and immunohistochemistry staining for LAG-3 and TIGIT might be valuable for screening patients suitable for immune checkpoint immunotherapy.

Dual blockade with PD-1 and LAG-3 antibodies results in a significant and consistent effect in terms of increased cytokine production by tumor antigen–specific $CD8^+$ T cells.[24,25] Preclinical data demonstrate that the effect of anti–LAG-3 monotherapy is mild, but the anti-tumor effect is synergized with PD-1 antibodies.[25]

There are limitations to this study. We conducted a single institution study, and patients are often referred to our center, creating the potential for selection bias in our study. Because the analyses in this study were exploratory, *P* values have not been adjusted for multiplicity and must be interpreted with caution. Additional dedicated studies are needed to confirm our results.

In conclusion, our study implicates that investigations regarding the expression of LAG-3 and TIGIT in melanoma might be of pivotal future interest. Expression of LAG-3 and TIGIT had prognostic value in cutaneous melanoma and could play a role in defining the function of PD-1 expression. LAG-3 and TIGIT inhibitors might be of particular use in combination with anti-immune stimulatory protocols involving PD-1 antibodies.

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