Investigation of LAG3 and PD1 in Melanoma Through Integrative Analysis of TCGA and GEO Data

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Take Home Messages

- High expression of *LAG3* and *PD1* was associated with improved overall survival in melanoma patients.
- IL2RG and JAK3 were identified as potential prognostic markers.
- Patients with *PD1+LAG3+JAK3+IL2RG* co-overexpression were more likely to respond well to anti-PD1 therapy than those with *PD1* overexpression alone.

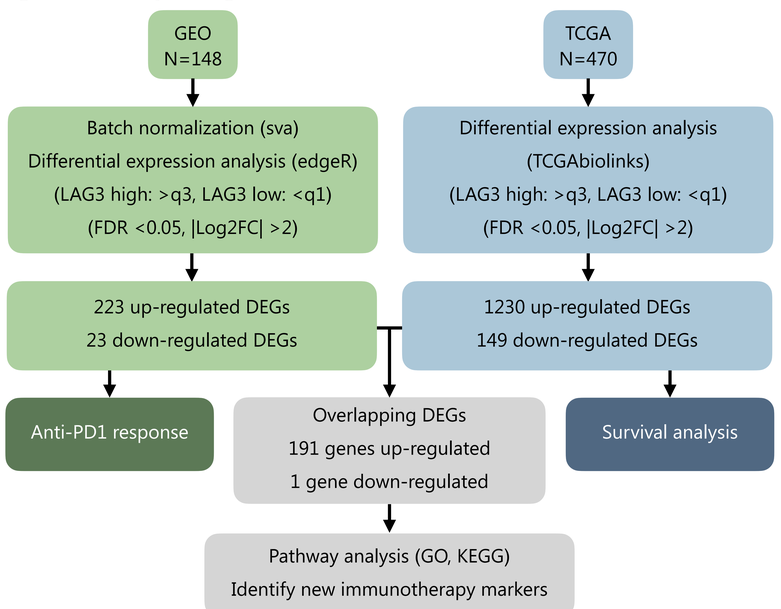
Objectives

Lymphocyte activation gene-3 (LAG3) has become a promising cancer immunotherapy target. This study aims to determine the synergistic effect of anti-LAG3 and anti-PD1 against melanoma and identify new potential targets for melanoma treatment.

Methods

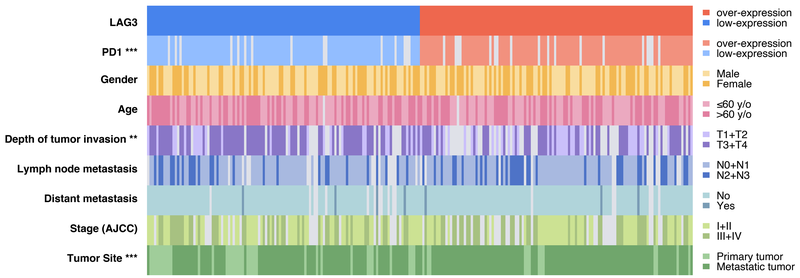
This study investigated the impact of *LAG3* and *PD1* expression levels on melanoma clinical manifestations, survival rates, and anti-PD1 treatment responses through an integrative analysis of The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) data. Differentially expressed genes (DEGs) enriched with *LAG3* were selected for pathway analysis to understand *LAG3*'s biological function and provide new insights for immunotherapy drug development. R version 4.0.2 was used for all statistical analyses.

Figure 1. Study Design



Results

Figure 2. Baseline characteristics of TCGA melanoma patients. *LAG3* expression was significantly correlated with *PD1* expression (p=1.42×10^{-60}), early-stage tumor invasion (T1+T2) (p=9.91×10^{-3}), and the metastatic tumor site (p=2.02×10^{-4}).



* p<0.05; **p<0.01; ***p<0.001

- 470 TCGA samples were categorized into *LAG3* overexpression (>q3; N=118) and *LAG3* low-expression (<q1; N=118).
- *PD1* overexpression (>q3; N=100) and *PD1* low-expression (<q1; N=103) were selected from 470 samples.

Figure 3. Survival outcome and anti-PD1 response in patients with different gene expression levels. **A-B,** Patients with high expression of *LAG3* and *PD1* exhibited improved overall survival (p=1.69×10^{-6}; p=1.64×10^{-5}). **C,** A significant association was found between *LAG3+PD1* co-overexpression group and improved overall survival (p=4.22×10^{-5}). **D-E,** Patients from GEO datasets were categorized into early-stage (stage I to M1b, N=39) and late-stage (M1c, N=91) melanoma groups. Anti-PD1 responders tend to have higher *LAG3* and *PD1* expression levels in early-stage melanoma (p=0.09341).

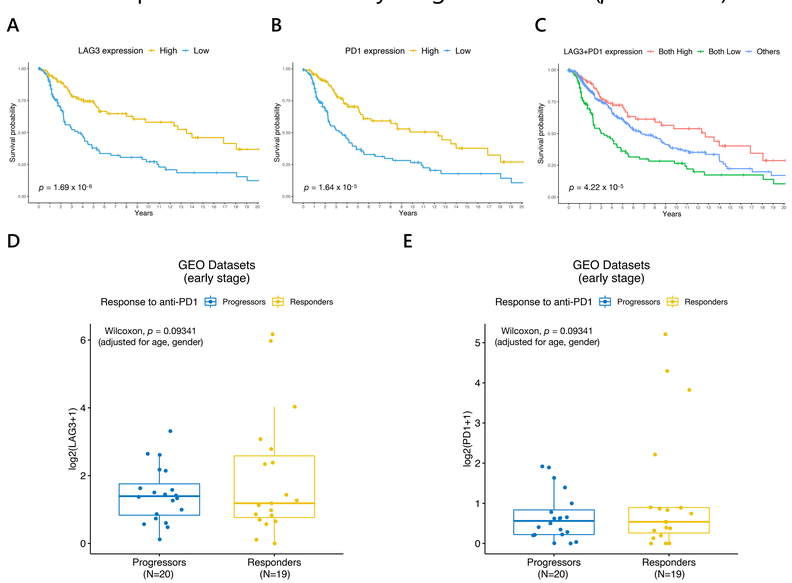


Figure 4. Pathway analysis of 191 overlapping up-regulated genes. **A,** DEGs enriched with *LAG3* were mainly involved in lymphocyte activation, proliferation, and differentiation. **B,** The KEGG pathway analysis revealed that primary immunodeficiency was the most significant pathway, with 12 genes enriched. **C-D,** Among the 12 genes, *JAK3* and *IL2RG* were targeted by approved drugs, and the co-overexpression of *JAK3* (p=0.082) and *IL2RG* (p=0.061) with *PD1* demonstrated a tendency towards improved overall survival.

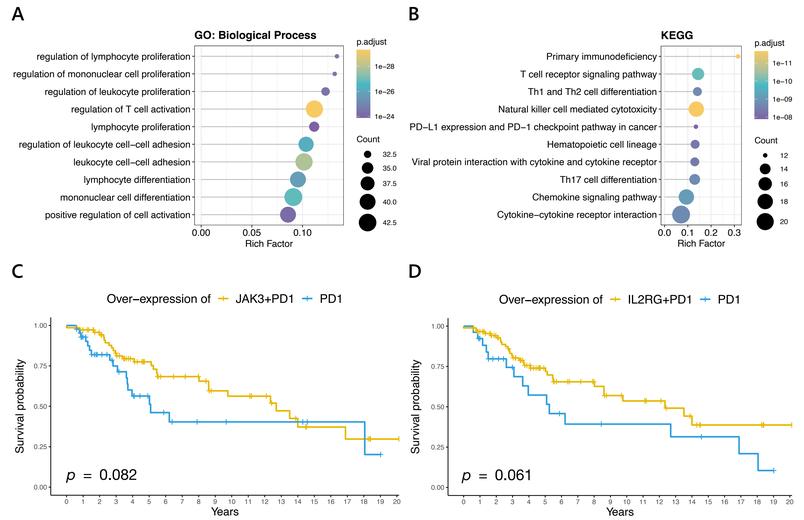


Table 1. Patients in the *PD1+LAG3+JAK3+IL2RG* overexpression group were more likely to respond well to anti-PD1 than patients with *PD1* overexpression alone (p=0.0092).

Characteristics	Total case N	Anti-PD1		p-value
		Progressors	Responders	
PD1+LAG3+JAK3+IL2RG	18	4	11	0.0092[a]
PD1 overexpression	19	11	3	

[a]p-value were calculated by Fisher's exact test.

Limitations and Conclusions

The cancer stage grouping method does not align with the clinical definition due to the uneven distribution of the GEO datasets. Moreover, a relatively small sample size was used to compare the difference in the anti-PD1 treatment response, which may reduce the power. Functional study of *JAK3* and *IL2RG* in melanoma is needed.

In summary, this study supports the anti-PD1 plus anti-LAG3 combination strategy for melanoma patients and highlights IL2RG and JAK3 as new therapeutic targets for preclinical investigation.

References: Shan C, Li X, Zhang J. Progress of immune checkpoint LAG-3 in immunotherapy. *Oncol Lett*. 2020;20(5):207. doi:10.3892/ol.2020.12070
Woo SR, Turnis ME, Goldberg MV, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res*. 2012;72(4):917-927. doi:10.1158/0008-5472.CAN-11-1620