Potential prognostic value of PD-L1 and NKG2A expression in Indonesian patients with skin nodular melanoma

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Research note

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Abstract

Objective

Biomarker mRNA levels have been suggested to be predictors of patient survival and therapy response in melanoma cases. This study aimed to investigate the correlations between the mRNA expression levels of PD-L1 and NKG2A in melanoma tissue and clinicopathologic characteristics and survival in Indonesian patients with primary nodular melanoma.

Results

Thirty-two tissue samples were analyzed. Upregulated PD-L1 was associated with shorter overall survival (hazard ratio: 2.930; 95% confidence interval: 1.011–8.489, \( p = 0.048 \)) compared with patients with normoregulated PD-L1. A significant positive correlation was found between the expression levels of PD-L1 and NKG2A (\( r_s = 0.768, p < 0.001 \)). However, no clinicopathologic associations with PD-L1 and NKG2A mRNA levels were statistically proven. Comparison with other studies suggested that the choice of adjuvant therapy and the presence of TILs affect the prognostic role of PD-L1 expression. NKG2A was not proven to be an independent predictive factor but may become an adjunct target for therapy. The strong correlation between PD-L1 and NKG2A suggests that anti-PD-1 and anti-NKG2A agents could be effective in patients with PD-L1 upregulation. The combination of the mRNA levels of these two target genes may provide a novel prognostic and therapeutic direction for immunotherapy.

Introduction

Among cutaneous malignancies, melanoma results in the highest mortality; statistics revealed 287,723 new cases and 60,712 deaths from this disease worldwide in 2018 [1]. These numbers are expected to increase, with an estimated 340,721 new cases projected in 2025 [2]. Despite trials of several combinations of systemic chemotherapeutic regimens, significant improvements in survival have not been achieved. Thus, current research is focused on new therapeutic agents, such as targeted therapy and immune checkpoint blockers.

Agents that block immune checkpoints, such as PD-1/PD-L1, help improve the immune response to cancerous cells. Nivolumab, an anti-PD-1 antibody, has been approved by the FDA for the treatment of advanced melanomas [3]. Other immunotherapies, such as monalizumab, a humanized anti-NKG2A antibody that enhances NK cell and CD8+ T cell activity [4], are also under development. These drugs show great promise and more durable responses compared with targeted therapy agents.

Before starting anti-PD-1 immunotherapy, clinicians commonly test tumor tissues for PD-L1 expression by using immunohistochemistry (IHC). Tumors expressing PD-L1 respond better to anti-PD-1/PD-L1 agents compared with non-expressers [5]. However, the results of recent studies on the effect of PD-L1 expression on survival are conflicting [6]. Detection using IHC also presents several limitations, such as the varying performance of different antibodies, nonstandard cut-off values, and operator dependence [7, 8].

Researchers are exploring new methods to predict therapy responses and survival in melanoma patients. Several studies have investigated the use of biomarker mRNA levels as an alternative parameter [9–11]. Gupta et al. reported that mRNA levels of PD-L1/2 show potential in predicting survival and response toward immunotherapy in metastatic melanoma [12]. Given the emergence of monalizumab, the potential prognostic and therapeutic roles of NKG2A should also be investigated.

Immuno-oncological research is fairly rare in Indonesia. Most Indonesian patients are treated with surgical resection and adjuvant treatment with dacarbazine chemotherapy and radiotherapy. Information on the expression of immune-checkpoint molecules is needed to gauge the potential efficacy of using immunotherapy agents in Indonesia. Thus, this study aimed to investigate the prognostic role of mRNA levels of PD-L1 and NKG2A, as well as the associated clinicopathologic characteristics.

Materials And Methods

Formalin-fixed paraffin-embedded (FFPE) tissue samples from patients diagnosed in 2012–2019 with primary cutaneous nodular melanoma were collected from the archives of the Department of Anatomical Pathology, Dr. Sardjito Hospital, which is the main cancer referral center in Yogyakarta, Indonesia. Cases with prior chemotherapy or radiotherapy, incomplete clinical data, and degraded specimens were excluded. Thirty-two samples were analyzed in this retrospective cohort study, and all patients were of Javanese ethnicity.

RNA was extracted from FFPE tissues using GeneAll™ Ribospin™ II (GeneAll Biotechnology, Seoul, South Korea). Real-time polymerase chain reaction (RT-PCR) for PD-L1 and NKG2A expression quantification was conducted using AccuPower® GreenStar™ RT-qPCR PreMix on an Exicycler™ 96 (Bioneer Corp., Daejeon, South Korea) with primer pairs and thermocycler conditions as previously described by Vassilakopoulou et al. [13, 14]. The expressions of PD-L1 and NKG2A were calculated from the quantification cycle (Cq) values of the gene targets and normalized against GAPDH as an internal control. Subsequent normalization was performed using the \( \Delta \Delta Cq \) values of RNA derived from healthy skin tissues. Age, sex, tumor location, Breslow thickness, greatest diameter, lymph node involvement, and stage were retrieved from medical records. Pathological data, including the presence of necrosis, lymphovascular invasion, tumor-infiltrating lymphocytes (TILs), and mitotic index, were obtained from hematoxylin–eosin and Ki67 IHC stained slides. Survival status (living or deceased) was determined through telephone calls at the point of follow-up of the study (until April 2020).

Samples were classified as normoregulated if the expression was lower than or equal to the mean of the PD-L1 and NKG2A levels; conversely, samples were classified as upregulated if the expression was above the mean of the PD-L1 and NKG2A levels. Comparison of mRNA level averages based on categorical
clinicopathologic characteristics was performed using Mann–Whitney U tests. Spearman correlation was used to analyze associations between the expression of PD-L1 and NKG2A and continuous clinicopathologic features. Kaplan–Meier analysis and log-rank tests with Cox regression were used to determine hazard ratios (HRs) for survival analysis.

Results

The characteristics of the subjects are presented in Table 1. Most tumors were located on the extremities (71.88%) and thicker than 4 mm (81.25%). Necrosis and TILs were present in 71.88% and 75% of the samples, respectively. The clinical stages were evenly distributed among stages II (31.25%), III (34.38%), and IV (34.38%).

The expression of PD-L1 and NKG2A was not significantly associated with the patients’ clinicopathologic characteristics (Additional file Table S1). Spearman correlation showed that NKG2A and PD-L1 mRNA levels were strongly correlated (Additional file Table S2).
Table 1
Clinicopathologic characteristics of the subjects

| Characteristic                        | Value          |
|--------------------------------------|----------------|
| Age (years), mean ± SD*              | 61.75 ± 16.28  |
| Sex, n (%)                           |                |
| Male                                 | 9 (28.12)      |
| Female                               | 23 (71.88)     |
| Tumor location, n (%)                |                |
| Trunk                                | 2 (6.24)       |
| Head and neck                        | 7 (21.88)      |
| Extremity                            | 23 (71.88)     |
| Lymph node metastases, n (%)         |                |
| Present                              | 21 (65.63)     |
| Absent                               | 11 (34.37)     |
| Breslow thickness                    |                |
| ≤ 1 mm                               | 0 (0.00)       |
| 1.01–2.00 mm                         | 3 (9.38)       |
| 2.01–4.00 mm                         | 0 (0.00)       |
| > 4.00 mm                            | 29 (90.62)     |
| Necrosis, n (%)                      |                |
| Present                              | 23 (71.88)     |
| Absent                               | 9 (28.12)      |
| Tumor diameter (mm), mean ± SD*      | 29.13 ± 24.20  |
| Ulceration                           |                |
| Present                              | 17 (53.13)     |
| Absent                               | 15 (46.87)     |
| Tumor-infiltrating lymphocyte        |                |
| Present (brisk and non-brisk)        | 24 (75.00)     |
| Absent                               | 8 (25.00)      |
| Clinical stage                       |                |
| I                                    | 0 (0.00)       |
| II                                   | 10 (31.25)     |
| III                                  | 11 (34.38)     |
| IV                                   | 11 (34.38)     |
| Survival status                      |                |
| Alive                                | 8 (25.00)      |
| Deceased                             | 24 (75.00)     |
| Overall survival (months), mean ± SD*| 22.81 ± 15.50  |

*SD: standard deviation

In the Cox univariate regression analysis, upregulated PD-L1 had an HR of 2.930 (95% CI: 1.011–1.963) for mortality compared with normoregulated cases (Additional file Table S3). Higher stage and NKG2A upregulation also increased HRs, but the differences observed were not statistically significant. In multivariate analysis, all three variables were weakly correlated.

Figure 1 shows that patients with normoregulated PD-L1 expression had significantly longer median survival time (27.000 ± 3.093 months) compared with subjects with upregulated expression (15.000 ± 3.657 months; p = 0.036). No significant difference in survival time was observed for the normoregulated (27.000 ± 3.221 months) and upregulated NKG2A subjects (19.000 ± 3.811 months; p = 0.483) (Fig. 2).
The survival curves of patients with and without TILs, which did not differ significantly ($p = 0.662$) (Additional file Fig. 1). The survival curves of the upregulated and normoregulated groups for PD-L1 and NKG2A did not differ significantly when divided based on the presence of TILs (Additional files Figs. 2 and 3).

**Discussion**

In this study, we compared the clinicopathologic characteristics and overall survival of Indonesian primary nodular melanoma cases with different mRNA levels of PD-L1 and NKG2A. Two important findings were observed in our study: (1) melanoma cases with PD-L1 overexpression had significantly lower survival rates compared with those with normal PD-L1 expression and (2) PD-L1 and NKG2A levels were strongly correlated.

We observed that patients with tumors showing upregulated PD-L1 had significantly lower overall survival, with an approximately threefold higher HR, compared with those with tumors showing normal PD-L1. The PD-L1 molecule interacts with PD-1 receptors on T cells, causing anergy, exhaustion, and even apoptosis [15]. Melanoma cells can thus escape the immune system by increasing their PD-L1 expression. Experiments using melanoma cell lines showed that cells with upregulated PD-L1 demonstrate highly invasive and aggressive behavior [16]. In a study on melanoma patients treated with surgery and dacarbazine adjuvant chemotherapy, patients with positive PD-L1 on IHC staining had lower median survival time compared with the subgroup with negative or indeterminate PD-L1 status (9.7 months vs. 11.6 months) [17].

Our results seem to contradict those of Gupta *et al.*, who observed that higher PD-L1 mRNA levels reflect a better prognosis for patients with melanoma treated with anti-PD-1 agents [12]. This incongruity may have stemmed from differences in the treatments administered to the subjects. Patients with high expression of PD-L1 respond well to anti-PD-1 antibodies, thus explaining the increase in progression-free and overall survival [5]. The results suggest that PD-L1 expression is a negative prognostic factor in patients with melanoma in general. However, when treated with anti-PD-1 antibodies, patients with high levels of PD-L1 respond well and have good outcomes. Therefore, the choice of therapy also affects the performance of PD-L1 as a prognostic factor.

PD-L1 expression can occur as an independent phenomenon or a secondary reaction to the presence of TILs. TILs can secrete interferon-gamma, which induces the expression of PD-L1 in tumor cells [15]. Studies have reported the reactive pathway as the predominant background for PD-L1 expression in melanomas because of the strongly positive correlation between PD-L1 and TILs [18]. However, in our study, the group without TILs had higher average PD-L1 mRNA levels than the group with TILs. Combined with the lower survival of PD-L1 overexpressers, this finding suggests that the cases with increased PD-L1 in our study likely have no TILs and are independent (constitutive) expressers.

Constitutive and reactive expression of PD-L1 may have different prognostic implications. When previous studies divided patients based on PD-L1 expression and the presence of TILs, patients with constitutive PD-L1 expression without lymphocyte infiltrates showed the poorest outcomes, followed by reactive PD-L1 expressers, those with PD-L1(−) without TILs, and, finally, those with PD-L1(+) and TILs [16, 19]. In our results, both groups with upregulated PD-L1 showed poorer prognoses than the groups with normoregulated PD-L1. However, survival did not differ significantly when the cases were divided further based on TIL status, likely because none of the patients were treated using immunotherapy, in which the presence of TILs predicts improved response and outcomes [20]. Another factor that could explain this lack of significance is the limited sample size.

Patients with upregulated NKG2A mRNA did not differ significantly from normo-regulated patients in terms of survival. NKG2A is an inhibitory receptor found on NK cells that plays a major role in the immune response against tumors [21]. Cancer cells can attempt to evade the immune system by upregulating HLA self-molecules that activate NKG2A receptors and impair the function of NK cells. Trials in mouse models indicate that monalizumab is ineffective as a single therapy but highly effective when used together with other immunotherapy agents that promote activated TILs, such as anti-PD-1 or cancer vaccines [22]. One escape strategy used by cells to escape cytotoxic TILs is downregulation of MHC I expression, which renders them targets for NK cells [23]. This finding may explain the role of anti-NKG2A as an adjunct treatment for other immunotherapies. Our results reinforce the idea that NKG2A may not be an independent therapeutic and prognostic factor but may play a role when combined with other factors.

The mRNA expressions of NKG2A and PD-L1 were strongly correlated. This finding indicates that tumors with high PD-L1 expression would also likely express NKG2A strongly and, thus, respond well to anti-NKG2A agents. When NKG2A expression was combined with the TIL parameter, the distribution of survival curves obtained resembled the curves for PD-L1 combined with TILs. NKG2A upregulation with and without the presence of TILs may have different pathogeneses and prognostic implications, similar to PD-L1.

The lack of correlation between the expression of NKG2A and PD-L1 and clinicopathologic characteristics in this work resembles the findings of several previous studies [6, 18]. The small sample size of this study may have contributed to the low statistical significance found.

The findings of this study must be interpreted with caution because of the small sample size of patients who did not receive adjuvant immunotherapy. However, our results support the findings of several studies that show that mRNA profiles may serve as a prognostic factor in melanoma cases [12, 24]. Our interesting results suggest that further research and clinical trials are warranted to ascertain the roles of PD-L1 and NKG2A in the prognosis and therapy of Asian patients who had not previously received immune checkpoint inhibitors.

**Conclusions**

We investigated the correlations between PD-L1 and NKG2A expression levels and clinicopathologic characteristics and survival in patients with primary nodular melanoma in Yogyakarta, Indonesia. Patients with upregulated PD-L1 expression had significantly shorter overall survival than those with normoregulated expression. PD-L1 and NKG2A mRNA levels were positively correlated.
Our findings suggest that the choice of therapy and presence of TILs may affect the prognostic role of PD-L1 expression. NKG2A was not proven to be an independent predictive factor but may serve as an adjunct target for therapy. The strong correlation between PD-L1 and NKG2A suggests that anti-PD1 and anti-NKG2A agents may be effective in patients with PD-L1 upregulation. Studies with larger subject groups are needed to confirm the patterns of PD-L1 expression in Asian cases.

**Limitations**

Our study was limited by its small sample size and homogenous ethnic population. Results among diverse Indonesian and Asian populations may differ. Examination of TILs did not discriminate between lymphocyte subtypes.

**Abbreviations**

CD
Cluster of differentiation
Cq
Quantification cycle
DNA
Deoxyribonucleic acid
FFPE
Formalin-fixed paraffin-embedded
GADPH
Glyceraldehyde 3-phosphate dehydrogenase
HLA
Human leukocyte antigen
IHC
Immunohistochemistry
MHC
Major histocompatibility complex
mRNA
Messenger ribonucleic acid
NK
Natural killer
NKG2A
Natural killer group 2A
PD-1
Programmed death-1
PD-L1
Programmed death-ligand 1
RT-PCR
Real-time polymerase chain reaction
TILs
Tumor-infiltrating lymphocytes

**Declarations**

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**Availability of data and materials**

This submission contains all of the data analyzed during the study. Unprocessed data can be requested from the corresponding author.

**Authors’ contributions**
RDS designed the study and performed the analysis. HTR, MFP, SLA, KS, and TA wrote the manuscript. YI and MRR contributed to the data collection. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study complied with the Declaration of Helsinki and the Belmont Report. The protocol for this study was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (KE/FK/0599/EC/2020). The study was conducted with formal permission from the appropriate hospital officials after explaining the research objectives and procedures. Patients were informed preoperatively of the use of data and tissue samples for research. All patients have consented in written forms.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. International Agency for Research on Cancer. GLOBOCAN fact sheet: melanoma of skin [Internet]. Global Cancer Observatory. 2018. Available from: http://globo.cancer.iarc.fr/old/bar_sex_site.asp?selection=16120&title=Melanoma+of+skin&statistic=2&populations=6&window=1&grid=1&color1=5&color2=4&submit=Execute.

2. International Agency for Research on Cancer. Estimated number of incident cases from 2018 to 2040: Melanoma of skin [Internet]. Cancer Tomorrow. 2018. Available from: https://gco.iarc.fr/tomorrow/graphic-line?type=0&type_sex=0&mode=population&sex=0&populations=900&cancers=16&age_group=value&pc_male=0&pc_female=0&single_unit=500000&print=0.

3. Domingues B, Lopes JM, Soares P, Pópulo H. Melanoma treatment in review. ImmunoTargets Ther. 2018;7:35–49.

4. Van Hall T, André P, Horowitz A, Ruan DF, Borst L, Zerbib R, et al. Monalizumab: inhibiting the novel immune checkpoint NKG2A. J Immunother Cancer. 2019;7(1):263.

5. Abdel-Rahman O PD. PD-L1 expression and outcome of advanced melanoma patients treated with anti-PD-1/PD-L1 agents: a meta-analysis. Immunotherapy. 2016;8(9):1081–9.

6. Yang J, Dong M, Shui Y, Zhang Y, Zhang Z, Mi Y, et al. A pooled analysis of the prognostic value of PD-L1 in melanoma: evidence from 1062 patients. Cancer Cell Int. 2020;20(1):96. https://doi.org/10.1186/s12935-020-01187-x.

7. Patel SP, Kurzrock R. PD-L1 expression as a predictive biomarker in cancer immunotherapy. Mol Cancer Ther. 2015;14(4):847–56.

8. Lee HH, Wang YN, Xia W, Chen CH, Rau KM, Ye L, et al. Removal of N-linked glycosylation enhances PD-L1 detection and predicts anti-PD-1/PD-L1 therapeutic efficacy. Cancer Cell Int. 2019;36(2):168–178.e4.

9. Tang Y, Xie C, Zhang Y, Qin Y, Zhang W. Overexpression of mRNA-decaping enzyme 1a predicts disease-specific survival in malignant melanoma. Melanoma Res. 2018;28(1):30–6.

10. Hoffmann F, Zarbl R, Niebel D, Sirokay J, Fröhlich A, Posch C, et al. Prognostic and predictive value of PD-L2 DNA methylation and mRNA expression in melanoma. Clin Epigenet. 2020;12(1):94.

11. Pan X, Liu R, Li Z. The prognostic value of HRAS mRNA expression in cutaneous melanoma. BioMed Res Int. 2017;2017:5356737.

12. Gupta S, McCann L, Chan YGY, Lai EW, Wei W, Wong PF, et al. Closed system RT-qPCR as a potential companion diagnostic test for immunotherapy outcome in metastatic melanoma. J Immunother Cancer. 2019;7(1):254.

13. Vassilakopoulou M, Avgeris M, Velchetti V, Kotoula V, Rampias T, Chatzopoulos K, et al. Evaluation of PD-L1 expression and associated tumor-infiltrating lymphocytes in laryngeal squamous cell carcinoma. Clin Cancer Res. 2016;22(3):704–13.

14. Meckawy GR, Mohamed AM, Zaki WK, Khattab MA, Amin MM, ElDeeb MA, et al. Natural killer NKG2A and NKG2D in patients with colorectal cancer. J Gastrointest Oncol. 2019;10(2):218–25.

15. Taube JM, Anders RA, Young GD, Xu H, Sharma R, McMullin TL, et al. Colocalization of inflammatory response with B7-H1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med. 2012;4(127):127ra37.

16. Audrito V, Serra S, Stingi A, Orso F, Gaudino F, Bologna C, et al. PD-L1 up-regulation in melanoma increases disease aggressiveness and is mediated through miR-17-5p. Oncotarget. 2017;8(9):15894–911.

17. Robert C, Long GV, Brady B, Dutriaux C, Di Giacomo AM, Mortier L, et al. Five-year outcomes with nivolumab in patients with wild-type BRAF advanced melanoma. J Clin Oncol. 2020;JCO.20.00995;38(33):3937–46.

18. Obeid JM, Erdag G, Smolkin ME, Deacon DH, Patterson JW, Chen L, et al. PD-L1, PD-L2 and PD-1 expression in metastatic melanoma: correlation with tumor-infiltrating immune cells and clinical outcome. Oncoimmunology. 2016;5(11):e1235107. http://doi.org/10.1080/2162402X.2016.1235107.

19. Massi D, Brusa D, Merelli B, Falcone C, Xue G, Carobbio A, et al. The status of PD-L1 and tumor-infiltrating immune cells predict resistance and poor prognosis in BRAFi-treated melanoma patients harboring mutant BRAFV600. Ann Oncol. 2015;26(9):1980–7. http://doi.org/10.1093/annonc/mdv255.

20. Badalamenti G, Fanale D, Incorvaia L, Barraco N, Listi A, Maragliano R, et al. Role of tumor-infiltrating lymphocytes in patients with solid tumors: can a drop dig a stone? Cell Immunol. 2019;343(October 2017):103753. https://doi.org/10.1016/j.cellimm.2018.01.013.
21. Zaghi E, Calvi M, Marcenaro E, Mavilio D, Di Vito C. Targeting NKG2A to elucidate natural killer cell ontogenesis and to develop novel immune-therapeutic strategies in cancer therapy. J Leukoc Biol. 2019;105(6):1243–51.

22. Borst L, van der Burg SH, van Hall T. The NKG2A–HLA-E axis as a novel checkpoint in the tumor microenvironment. Clin Cancer Res. 2020;26(21):5549–56.

23. Lee H, Quek C, Silva I, Tasker A, Batten M, Rizos H, et al. Integrated molecular and immunophenotypic analysis of NK cells in anti-PD-1 treated metastatic melanoma patients. Oncoimmunology. 2019;8(2):e1537581. https://doi.org/10.1080/2162402X.2018.1537581.

24. Jayawardana K, Schramm SJ, Haydu L, Thompson JF, Scolyer RA, Mann GJ, et al. Determination of prognosis in metastatic melanoma through integration of clinico-pathologic, mutation, mRNA, microRNA, and protein information. Int J Cancer. 2015;136(4):863–74.

Figures

Figure 1

Kaplan–Meier survival curves comparing the survival of patients with primary nodular melanoma with upregulated or normoregulated PD-L1 expression (p = 0.036)

Figure 2

Kaplan–Meier survival curves comparing the survival of patients with primary nodular melanoma with upregulated or normoregulated NKG2A expression
Kaplan–Meier survival curves comparing the survival of patients with primary nodular melanoma with upregulated or normoregulated NKG2A expression (p = 0.483)

**Supplementary Files**

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