Heliyon 8 (2022) e12648

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

A pretreatment transcriptomic signature that predicts outcomes of immunotherapy in melanoma

Junjie Hu [a], Bei Liu [a], Wangxiong Hu [b,*], Yanmei Yang [a,**]

[a] Key Laboratory of Reproductive and Genetics, Ministry of Education, Women's Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310006, China
[b] Cancer Institute (Key Laboratory of Cancer Prevention and Intervention, China National Ministry of Education), The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310009, China

ARTICLE INFO

Keywords:
Immune checkpoint inhibitor
irRECIST
Melanoma
PD-1
Risk score

ABSTRACT

Identifying indicators of immunotherapy response are key to clinical treatment decisions. To date, immunotherapy is most widely used in melanoma because of its higher tumor mutation burden compared to other cancer types. However, less than half of melanoma patients can benefit from immune checkpoint inhibitor (ICI) therapy. For this reason, we deciphered pretreatment transcriptomes across a cohort of melanoma patients receiving anti-PD-1 or CTLA-4 alone (sICI) or in combination (cICI). We developed a two-gene signature that could predict the curative effect of ICI in melanoma by using the LASSO method. The pre-ICI signature displayed an equally competitive predictive power as the post-ICI irRECIST assessment that could offer clues regarding long-term ICI therapy response and facilitate risk stratification and treatment strategies.

1. Introduction

Substantial success has been achieved by immune checkpoint inhibitor (ICI) therapy in melanoma and other cancer types in the last few years [1]. In particular, the blockade of cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed cell death receptor 1 (PD-1) are therapeutic paradigms that have revolutionized the oncotherapy because of their excellent antitumor ability [2]. In melanoma, despite the high response rate due to its relatively high tumor mutation burden (TMB), many patients are refractory to therapy or acquire resistance [3]. Thus, the identification of factors or gene-set signatures that effectively predict responses to ICI is urgently required for grasping and expanding the use of ICI in melanoma. Litchfield et al. [4] found CCR5 and CXCL13 were T-cell-intrinsic markers of ICI sensitivity. However, there are few studies on the establishment of pretreatment signatures that can predict ICI response.

Here, we constructed a pretreatment transcriptomic signature based on RNAseq that can predict ICI sensitivity (anti-PD-1 antibodies (nivolumab or pembrolizumab) or anti-CTLA-4 antibody ipilimumab) in PRJEB23709 and TCGA cohorts. Then, the predictive power of the signature was validated in another dataset including two independent cohorts with 79 advanced melanoma samples who received ICI therapy. We found that the two-gene signature had equal predictive power with post-treatment irRECIST (Immune-related Response Evaluation Criteria In Solid Tumors)-derived responders (defined as complete response [CR] and partial response [PR]) compared to nonresponders (defined as stable disease [SD] and progressive disease [PD]).

This study aimed to develop a robust and clinical-friendly prognostic profile that can be applied to predict the treatment effect of anti-PD-1 or anti-CTLA-4 immunotherapy and to better select patients who are resistant for alternative therapeutic manipulation in melanoma.

2. Materials and methods

2.1. Sample collection and selection

A systematic search to collect publicly available datasets was carried out in January 2021 using the key word "melanoma" and filtering to retain only human samples subjected to expression profiling experiments in multiple known repositories. Datasets with fewer than 10 samples and clinical trials not related to ICIs for melanoma were excluded. Finally, only the expression profiles containing pretreatment

* Corresponding author.
** Corresponding author.
E-mail addresses: wxhu@zju.edu.cn (W. Hu), yangyanmei@zju.edu.cn (Y. Yang).

https://doi.org/10.1016/j.heliyon.2022.e12648
Received 25 May 2022; Received in revised form 15 November 2022; Accepted 19 December 2022

samples were kept for further analysis. This study was approved by the Ethics Committee of the Second Affiliated Hospital, Zhejiang University School of Medicine. Informed consent was obtained from all patients and this study was conducted in accordance with the Declaration of Helsinki.

2.2. Gene expression data processing and normalization

There were four datasets enrolled in this study. The GSE78220 [5] and GSE91061 [6] datasets were retrieved from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). The raw fastq sequencing data of PRJEB23709 [7] were downloaded from the European Nucleotide Archive (ENA, https://www.ebi.ac.uk/ena). Then, the paired-end reads were mapped to human protein coding genes (Homo_sapiens.GRCh38.84) using HISAT2 (version 2.2.0) with the default parameters [8] and counted by featureCounts [9]. Level 3 tumor RNASeqV2 mRNA datasets were downloaded from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/) [10]. Approximately 3,000 genes were removed because of their expression levels <1 in more than 50% of samples. Batch effects were corrected with the function *ComBat* in the Bioconductor package *sva* [11].

2.3. Survival analysis

A batch Cox univariate model of the ~16000 genes was performed with the *coxph* function in the *survival* package in R. A P value <0.05 was considered significant. Then, genes that were significantly correlated with melanoma patient survival were further shrunk using the least absolute shrinkage and selection operator (LASSO) regression algorithm. The best λ was determined by 10-fold cross-validation using the built-in function *cv. glmnet* in *glmnet* package [12]. The patients were divided into high- and low-risk groups by calculating the prognostic index (PI) as follows:

$$PI_k = \sum_{g=1}^{n} \beta_g m_{gk}$$

where n is the number of significantly survival-related genes, β_g is the regression coefficient of the Cox proportional hazard model for gene g, and m_{gk} is the expression level of gene g in patient k. Melanoma patients were then classified into high- and low-risk groups based on the median PI value. The survival difference between the two groups (high- and low-risk) was analyzed with the log-rank test with functions *survfit* and *survdiff* in the R package *survival* [13]. The hazard ratio (HR) of the high-risk group versus the low-risk group was determined by the proportional hazards model. A P value <0.05 was considered significant.

2.4. Assessment of signature predictive power in response to ICI therapy

To decipher the predictive power of our signature in melanoma ICI therapy, the irRECIST-based curative effect after ICI therapy was compared between the high- and low-risk groups. Briefly, ‘‘responder’’ was defined as complete response (CR) or partial response (PR), and ‘‘nonresponder’’ was defined as stable disease (SD) or progressive disease (PD). Then, the proportion of responders was compared between the low- and high-risk groups as defined by the tailored signature based on the expression profiles.

2.5. Data and code availability

The codes used in this study are publicly accessible at (https://github.com/huwangxiong/A-pre-treatment-transcriptomic-signature-that-predicts-outcomes-of-immunotherapy-in-melanoma). All other data are available from the authors upon reasonable request.

3. Results

3.1. Profiling of expression-based melanoma patient response to immunotherapy with CTLA-4 and PD-1 blockade

Our study aimed to identify a transcriptomic signature that could be used for the early prediction of melanoma patient outcomes before ICI (mainly anti-PD-1 or anti-CTLA-4 antibodies) therapy. Thus, we only consider the samples before ICI treatment. In total, four independent cohorts (i.e., GSE78220, 28 pretreatment tumors with pembrolizumab or nivolumab as the anti-PD-1 therapy; GSE91061, 51 pretreatment tumors with nivolumab; PRJEB23709, 75 pretreatment tumors with pembrolizumab or nivolumab or combined anti-CTLA-4 and anti-PD-1 therapy (nivolumab or pembrolizumab combined with ipilimumab); and TCGA, 23 pretreatment tumors with ipilimumab) consisting of 177 samples were enrolled in this study (Table S1).

3.2. Establishment of a signature that can predict melanoma clinical outcomes in advance of ICI

To screen the genes that are significantly associated with melanoma patient survival, batch univariate Cox regression was performed for the 16,218 genes. Notably, 759 genes were found to be significantly correlated with melanoma patient outcome (Fig. S1, likelihood ratio test, $P <$ 0.05). Then, the 759 genes were subjected to LASSO analysis to shrink the parameters in the final signature. A well-conceived two-gene signature (DYNLL1 and OSBPL10) was identified that could powerfully predict the outcomes of melanoma patients.

Notably, we found that melanoma patients can be divided into two groups according to the LASSO-derived signature with the formula as: risk score (RS) = DYNLL1*2.677e^{-05}-OSBPL10*7.645e^{-05}. The coefficients in the signature were determined based on the optimal lambda value in the LASSO model. The high-risk group was significantly correlated with unfavorable OS in the TCGA & PRJEB23709 cohorts when compared with the low-risk group (two-year survival, 0.54 vs. 0.87, 95% confidence interval (CI) 0.41–0.71 vs. 0.78–0.97; Figure 1A). Furthermore, the high-risk group had significantly shorter disease-free survival (DFS) than the low-risk group (log rank test P = 0.001; Figure 1B). Undoubtedly, the low-risk group had a lower risk than the high-risk group, with a hazard ratio (HR) of 4.813e+09 (95% CI = 121.8–1.902e+17, Wald test P = 0.01) in the TCGA & PRJEB23709 cohorts, with a two-year survival of 0.58 vs. 0.35 (95% CI 0.45–0.75 vs. 0.23–0.53; Figure 1B). The finding was validated in independent GSE78220 and GSE91061 cohorts (log rank test P = 4e-04; Figure 1C), with two-year survival rates of 0.29 and 0.61 (95% CI 0.17–0.49 and 0.47–0.80) in the high- and low-risk groups, respectively.

3.3. Two-gene derived signature positively correlated with irRECIST in monitoring of ICIs in melanoma

iRECIST is regarded as the gold standard for response assessment of oncologic patients under immunotherapy. As mentioned earlier, our signature was an important independent prognostic factor, and whether the low-risk group was more related to the ICI response in melanoma aroused our curiosity. To ensure consistency in outcome assessment, ‘‘responder’’ was defined as irRECIST-based response with complete response (CR) or partial response (PR) and ‘‘nonresponder’’ was defined as stable disease (SD) or progressive disease (PD). Approximately twofold more responders were found in the low-risk group than in the high-risk group in the TCGA and PRJEB23709 discovery cohorts (Figure 2A). Especially for the CR patients, fourfold more patients were belonged to the low-risk group after PD1-or CTLA-4-targeted ICI therapy. Similar trends were observed in the GSE78220 and GSE91061 validation cohorts (Figure 2B). We thus further separately compared the HR between our signature and irRECIST criteria in melanoma patients who received anti-CTLA-4 and anti-PD-1 immunotherapy. Intriguingly, our signature had a similar prediction power as after treatment of ICI therapy evaluated by

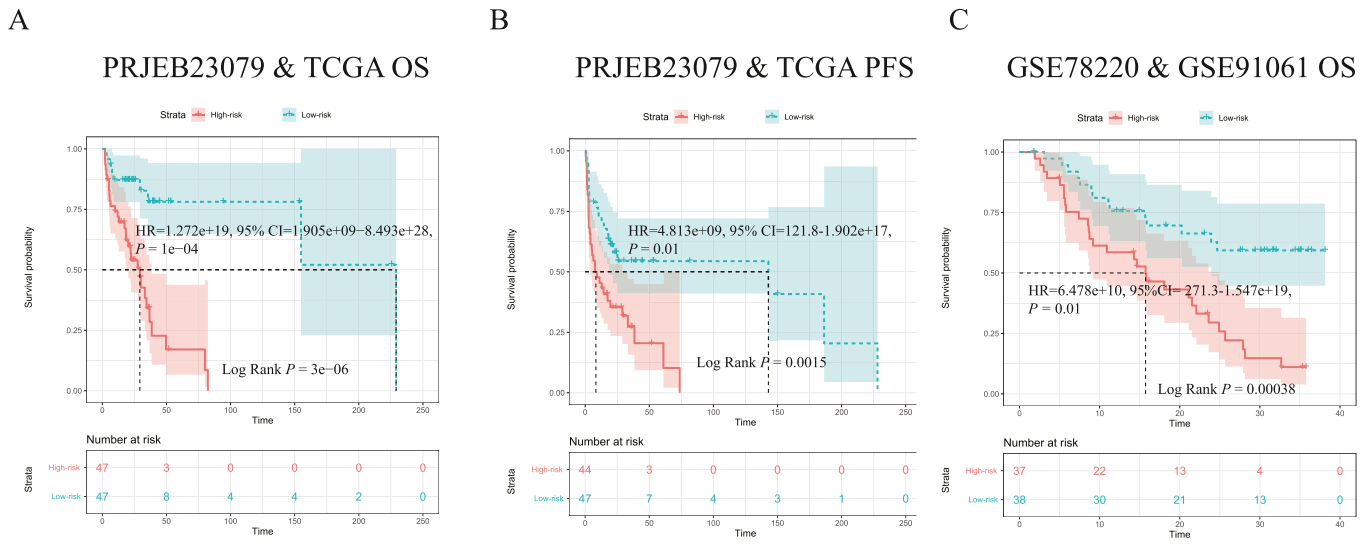

Figure 1. The survival differences between the low- and high-risk groups based on the two-gene signature in melanoma patients. **A.** KM plot of OS status for samples from PRJEB23079 and TCGA. **B.** KM plot of PFS status for samples from PRJEB23079 and TCGA. **C.** KM plot of OS status for samples from GSE78220 and GSE91061.

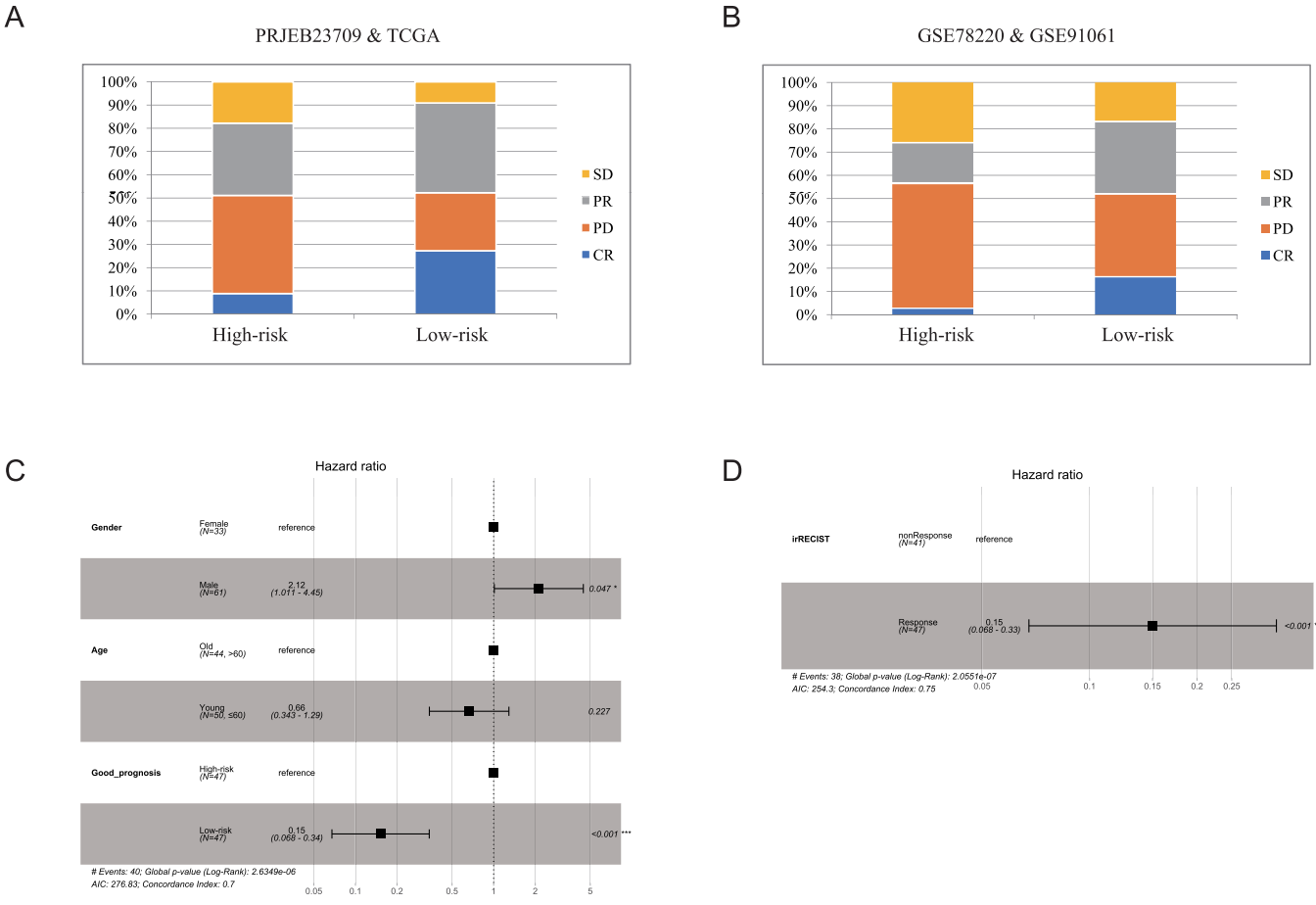

Figure 2. The association of two-gene derived risk groups and irRECIST-based response in melanoma. **A.** The distribution of CR, PD, PR, and SD determined by irRECIST was visualized by histogram according to low- and high-risk groups in the PRJEB23079 and TCGA cohorts. **B.** The distribution of CR, PD, PR, and SD determined by irRECIST was visualized by histogram according to low- and high-risk groups in the GSE78220 and GSE91061 cohorts. **C.** Multivariate Cox PH analysis including the risk score, age, and sex of melanoma patients from the PRJEB23079 and TCGA cohorts. **D.** A forest figure of Cox PH analysis based on the irRECIST responders compared to non-responders from the PRJEB23079 and TCGA cohorts.

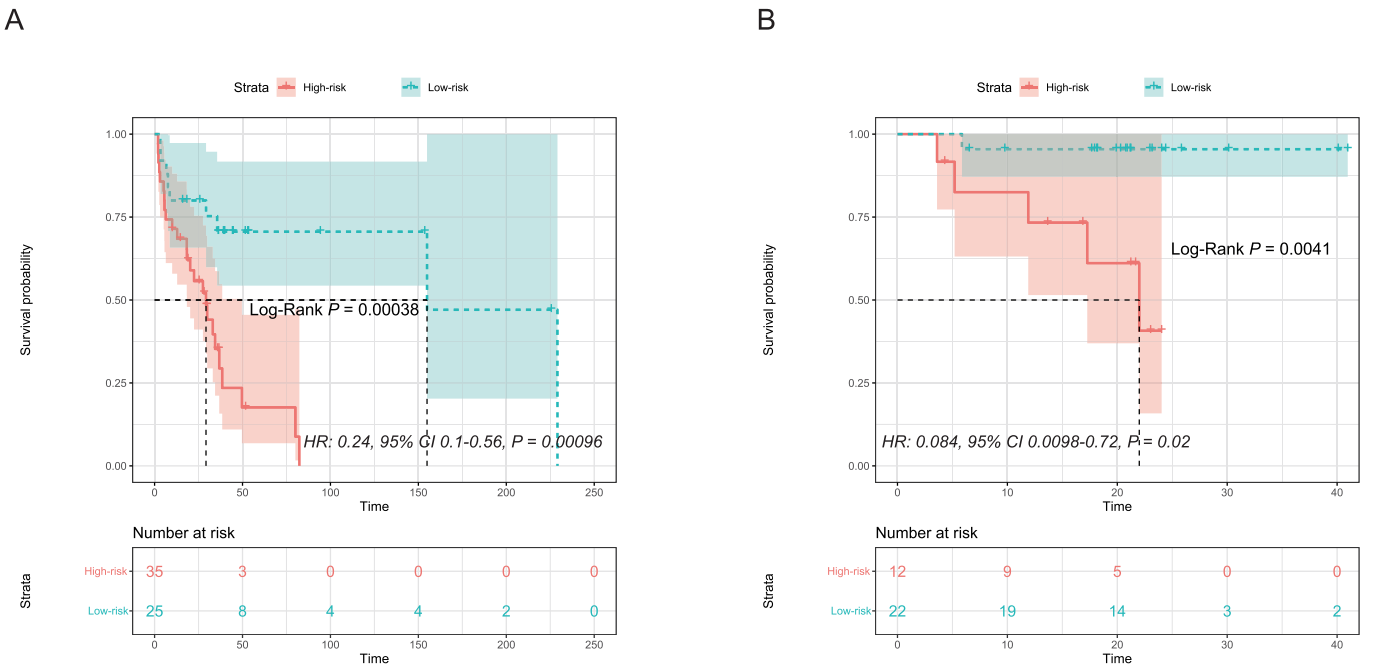

Figure 3. The survival differences between the low- and high-risk groups according to melanoma patients who received different therapeutic schedules. **A.** KM plot of OS status for samples from PRJEB23079 and TCGA who received a single ICI. **B.** KM plot of OS status for samples from PRJEB23079 and TCGA who received combinational ICI.

irRECIST criteria (low-risk group vs. high-risk group, HR: 0.15 (95% CI 0.068–0.34) vs. 0.15 (95% CI 0.068–0.33), Figure 2C and D), suggesting the robustness of the two-gene expression signature.

In addition, we explored the prediction power of our signature in the individual ICI group (pembrolizumab/ipilimumab/nivolumab) and the combined treatment group (ipilimumab + pembrolizumab/ipilimumab + nivolumab). Our signature clearly showed that the low-risk group had a significantly lower risk than the high-risk group in both the individual ICI group (HR: 0.24, 95% CI 0.1–0.56, Wald test $P = 0.00096$, Figure 3A) and the combined treatment group (HR: 0.084, 95% CI 0.0098–0.72, Wald test $P = 0.02$, Figure 3B).

4. Discussion

The success of cancer immunotherapy over the past five years has revolutionized routine clinical cancer treatment, especially for melanoma, which commonly has a high TMB [1]. Another compelling niche in ICI lies in the potential intrinsic immune cells infiltrated in tumor tissue. Most studies demonstrated that a higher CD8 T-cell infiltration within tumor tissue was the key factor that correlated with a good response to ICI [1]. Recently, Fairfax et al. [14] and Valpione et al. [15] reported that early changes (at 3 weeks) in T-cell repertoires via TCR sequencing and CD8+ memory effector cytotoxic T cells determined by flow cytometry in peripheral blood are closely associated with the response to ICIs and may serve as feasible biomarkers of immune activation in metastatic melanoma. However, biomarkers derived from pretreatment biopsy that can predict the long-term outcomes of the ICIs may be a more promising strategy for clinical therapeutics because early prediction brings benefits if patients are vulnerable to drug resistance. In this study, we identified a two-gene signature derived from pre-treatment tumor tissue via LASSO regression that can predict melanoma patient outcome when subjected to ICI (both anti-PD-1 or anti-CTLA-4 separately and in combination) therapy (Figure 3). This may substantially improve the therapeutic regimen if the sample belongs to the high-risk group. The LASSO Cox regression model has been widely used in predicting outcomes for tumor patients receiving diverse therapies by separating subjects into subgroups with high- and low-risk scores. For example, there is a seven-gene prognostic signature in osteosarcoma [16], a 20-gene diagnostic signature in gynecologic cancer [17], a prognostic eight-gene signature in breast cancer patients who receive adjuvant chemotherapy [18], a risk model composed of 10 immune-related genes in colon cancer [19], a six-gene prognostic signature in bladder cancer [20], and a nine-gene prognostic signature in gastric cancer [21]. In this study, DYNLL1 in our model was identified as a 53BP1 effector in response to DNA double-strand breaks and regulates checkpoint activation [22]. For OSBPL10, Chou et al. [23] recently clarified the potential prognostic role in patients with pancreatic ductal adenocarcinoma. These observations indicate that our signature is significantly associated with tumor progression. High-risk melanoma patients would benefit from an alternative combinational therapeutic schedule or other relatively novel immune checkpoint inhibitors such as targeting LAG3 and TIM3 instead of PD1 or CTLA-4 [24,25].

We recognize that there are some limitations in our current study. For instance, three different ICIs were adopted for melanoma patients, which may cause result bias. Additionally, the sample size of melanoma patients receiving ICI was limited by the difficulties of valuable data collection. We seek to collect more samples from patients who received ICI therapy to validate our conclusions. Furthermore, the precise mechanism of how the high-risk group was resistant to ICI therapy in melanoma remains to be elucidated. Related experiments, such as $CD8^+$ T-cell status determination and antitumor molecule, such as GZMB, PRF1 and IFN-γ detection, warrant further investigation.

In summary, we present a prognostic transcriptomic signature across cohorts of patients with melanoma receiving ICI. We show that the performance of this signature emulates the irRECIST rule in the assessment of the effect of immunotherapeutic agents (Figure 2C and D). This study illustrates the feasibility and power of using LASSO regression based on transcriptomics across different cohorts to discover long-sought predictive markers response to ICI therapy in melanoma.

Declarations

Author contribution statement

Junjie Hu: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Bei Liu: Analyzed and interpreted the data; Wrote the paper.

Wangxiong hu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Yanmei Yang: Conceived and designed the experiments; Wrote the paper.

Funding statement

Dr. Wangxiong hu was supported by National Natural Science Foundation of China [81802883].

Data availability statement

Data associated with this study are available from The Cancer Genome Atlas and Gene Expression Omnibus databases.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2022.e12648.

Acknowledgements

We thank ENA, GEO, and TCGA databases for supplying the valuable datasets.

References

[1] A.D. Waldman, J.M. Fritz, M.J. Lenardo, A guide to cancer immunotherapy: from T cell basic science to clinical practice, Nat. Rev. Immunol. 20 (11) (2020) 651–668.

[2] D.A. Braun, Z. Bakouny, L. Hirsch, R. Flippot, E.M. Van Allen, C.J. Wu, T.K. Choueiri, Beyond conventional immune-checkpoint inhibition - novel immunotherapies for renal cell carcinoma, Nat. Rev. Clin. Oncol. 18 (4) (2021) 199–214.

[3] D. Liu, J.R. Lin, E.J. Robitschek, G.G. Kasumova, A. Heyde, A. Shi, A. Kraya, G. Zhang, T. Moll, D.T. Frederick, et al., Evolution of delayed resistance to immunotherapy in a melanoma responder, Nat. Med. 27 (2021).

[4] K. Litchfield, J.L. Reading, C. Puttick, K. Thakkar, C. Abbosh, R. Bentham, T.B.K. Watkins, R. Rosenthal, D. Biswas, A. Rowan, et al., Meta-analysis of tumor- and T cell-intrinsic mechanisms of sensitization to checkpoint inhibition, Cell 184 (3) (2021) 596–614 e14.

[5] W. Hugo, J.M. Zaretsky, L. Sun, C. Song, B.H. Moreno, S. Hu-Lieskovan, B. Berent-Maoz, J. Pang, B. Chmielowski, G. Cherry, et al., Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma, Cell 165 (1) (2016) 35–44.

[6] N. Riaz, J.J. Havel, V. Makarov, A. Desrichard, W.J. Urba, J.S. Sims, F.S. Hodi, S. Martin-Algarra, R. Mandal, W.H. Sharfman, et al., Tumor and microenvironment evolution during immunotherapy with nivolumab, Cell 171 (4) (2017) 934–949, e16.

[7] T.N. Gide, C. Quek, A.M. Menzies, A.T. Tasker, P. Shang, J. Holst, J. Madore, S.Y. Lim, R. Velickovic, M. Wongchenko, et al., Distinct immune cell populations define response to anti-PD-1 monotherapy and anti-PD-1/anti-CTLA-4 combined therapy, Cancer Cell 35 (2) (2019) 238–255 e6.

[8] D. Kim, J.M. Paggi, C. Park, C. Bennett, S.L. Salzberg, Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype, Nat. Biotechnol. 37 (8) (2019) 907–915.

[9] Y. Liao, G.K. Smyth, W. Shi, featureCounts: an efficient general purpose program for assigning sequence reads to genomic features, Bioinformatics 30 (7) (2014) 923–930.

[10] T.C.G.A. Network, Genomic classification of cutaneous melanoma, Cell 161 (7) (2015) 1681–1696.

[11] W.E. Johnson, C. Li, A. Rabinovic, Adjusting batch effects in microarray expression data using empirical Bayes methods, Biostatistics 8 (1) (2007) 118–127.

[12] J. Friedman, T. Hastie, R. Tibshirani, Regularization paths for generalized linear models via coordinate descent, J. Stat. Software 33 (1) (2010) 1–22.

[13] T.M. Therneau, P.M. Grambsch, Modeling Survival Data: Extending the Cox Model, Springer, New York, 2000.

[14] B.P. Fairfax, C.A. Taylor, R.A. Watson, I. Nassiri, S. Danielli, H. Fang, E.A. Mahe, R. Cooper, V. Woodcock, Z. Traill, et al., Peripheral CD8(+) T cell characteristics associated with durable responses to immune checkpoint blockade in patients with metastatic melanoma, Nat. Med. 26 (2) (2020) 193–199.

[15] S. Valpione, E. Galvani, J. Tweedy, P.A. Mundra, A. Banyard, P. Middlehurst, J. Barry, S. Mills, Z. Salih, J. Weightman, et al., Immune-awakening revealed by peripheral T cell dynamics after one cycle of immunotherapy, Nat. Can. (Que.) 1 (2) (2020) 210–221.

[16] Z. Liu, Y. Zhong, S. Meng, Q. Liao, W. Chen, Identification of a seven-gene prognostic signature using the gene expression profile of osteosarcoma, Ann. Transl. Med. 10 (2) (2022) 53.

[17] S.H. Yu, J.H. Cai, D.L. Chen, S.H. Liao, Y.Z. Lin, Y.T. Chung, J.J.P. Tsai, C.C.N. Wang, LASSO and bioinformatics analysis in the identification of key genes for prognostic genes of gynecologic cancer, J. Personalized Med. 11 (11) (2021).

[18] Q. Cui, J. Tang, D. Zhang, D. Kong, X. Liao, J. Ren, Y. Gong, C. Xie, G. Wu, A prognostic eight-gene expression signature for patients with breast cancer receiving adjuvant chemotherapy, J. Cell. Biochem. 121 (6) (2019).

[19] X. Li, D. Wen, C. Yao, W. Chong, H. Chen, Identification of an immune signature predicting prognosis risk and lymphocyte infiltration in colon cancer, Front. Immunol. 11 (2020) 1678.

[20] F. Xu, Q. Tang, Y. Wang, G. Wang, K. Qian, L. Ju, Y. Xiao, Development and validation of a six-gene prognostic signature for bladder cancer, Front. Genet. 12 (2021), 758612.

[21] J. Chu, N. Sun, W. Hu, X. Chen, N. Yi, Y. Shen, Bayesian hierarchical lasso Cox model: a 9-gene prognostic signature for overall survival in gastric cancer in an Asian population, PLoS One 17 (4) (2022) e0266805.

[22] K.L. West, J.L. Kelliher, Z. Xu, L. An, M.R. Reed, R.L. Eoff, J. Wang, M.S.Y. Huen, J.W.C. Leung, LC8/DYNLL1 is a 53BP1 effector and regulates checkpoint activation, Nucleic Acids Res. 47 (12) (2019) 6236–6249.

[23] C.W. Chou, Y.H. Hsieh, S.C. Ku, W.J. Shen, G. Anuraga, H.D. Khoa Ta, K.H. Lee, Y.C. Lee, C.H. Lin, C.Y. Wang, et al., Potential prognostic biomarkers of OSBPL family genes in patients with pancreatic ductal adenocarcinoma, Biomedicines 9 (11) (2021).

[24] R. Derynck, S.J. Turley, R.J. Akhurst, TGFbeta biology in cancer progression and immunotherapy, Nat. Rev. Clin. Oncol. 18 (1) (2021) 9–34.

[25] D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy, Nat. Rev. Cancer 12 (4) (2012) 252–264.