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Identification of the transcriptomic alterations of resistance to immune checkpoint inhibitors in Melanoma

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Abstract. Although immunotherapeutics like immune checkpoint inhibitor (ICI) therapy have greatly improved survival rates, death rates of melanoma still remain high. One of the reasons for this is that the solid tumor microenvironment creates obstacles for the effectiveness of anti-PD1 immunotherapy in patients. Therefore, it is crucial to identify potential biomarkers that could be used in combination therapy with anti-PD1 to modify the tumor microenvironment and enhance response to treatment. In this study, we examined clinical and tumor transcriptional sequencing data from 91 patients who received anti-PD1/anti-CTLA4 therapy. Through both bulk RNA sequencing analysis and single-cell RNA-sequencing (scRNA-seq), we discovered that 8 key pathways were upregulated in patients who responded well to the therapy. Interestingly, these pathways were found in myeloid and T cell populations, indicating their significant role in response to anti-PD1/anti-CTLA4 therapy. Among these pathways, genes such as IRF1, IRF2, C1, and C3 emerged as potential biomarkers that could potentially enhance the effectiveness of ICIs therapy. Further clinical research is required to validate the impact of these genes. The novelty of this study lies in the combination of bulk RNA sequencing and single RNA sequencing methods, which allowed us to uncover distinct differences in the transcriptomic landscape of solid tumors, particularly melanoma.

Keywords: Anti-PD1, Immune Checkpoint Inhibitors, Melanoma

1. Introduction

Melanoma is the 17th most common cancer worldwide with estimated number of new patients to be around 325,000 in 2022 [1, 2]. Roughly 57,000 individuals lost their lives because of melanoma in 2020. Assuming the same yearly growth rate, there will be 510,000 new cases (around a 50% increase) and 96,000 deaths each year (a 68% increase) [1,3].

Immunotherapy has shed positive light on the treatment of advanced melanoma. For example, immune checkpoint inhibitors have significantly prolonged the median survival of patients with advanced inoperable stage IV diseases, from around 6 months to nearly 6 years [1]. In particular, anti-PD1 immunotherapies can achieve great control of melanoma progression, even producing a higher survival rate than chemotherapy [4, 5]. However, it remains unsatisfactory that current therapies are only effective to a fraction of patients, with an overall response rate of around 50% [6]. This failure is largely due to a suppressive tumor microenvironment which inhibits T-cell activity and support tumor progression [7].

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It is shown that genetic mutations are correlated with anti-PD1 therapy responses in non-small cell lung cancers, which could provide a potential indicator for anti-PD1 immunotherapy responses in melanoma [8]. However, mutations within tumors could be completely random because of chromatin instability, so genes are better reporters to predict anti-PD1 therapy response [5]. Compared to recent bulk RNA-sequencing studies in this area [9, 10], single-cell RNA sequencing (scRNA-seq) could provide higher resolution in transcriptomic landscape that may not be apparent in previous studies. Here, we try to reveal key genomic planning targets that may be used for rewriting the tumor immune environment to provide maximal benefit for solid cancer patients, enhancing anti-cancer immunotherapies in melanoma using both bulk-RNA and single-cell RNA sequencing (scRNA-seq).

2. Methodology

2.1. Patient Data and Databases

The data for our study was obtained from 91 melanoma patients who had undergone treatment with anti-PD1/anti-CTLA4 therapy. Of these patients, 73 had their transcriptional sequences collected before treatment, while 18 had sequences collected during the early stages of treatment. To assemble our dataset, we sourced the information from the Tumor Immunotherapy Gene Expression Resource (TIGER) database. In addition to the clinical and tumor transcriptional sequencing data, we also included single cell RNA-sequencing data from 31 patients with skin cutaneous melanoma who exhibited a positive response to anti-PD1 therapy. This data was obtained from the Tumor Immune Single-cell Hub 2 (TISCH2) database. This additional dataset allowed us to explore the transcriptional profiles of individual cells within these patients and gain insight into the mechanisms underlying the positive response to anti-PD1 therapy in melanoma.

By combining clinical and tumor transcriptional sequencing data from TIGER with the single cell RNA-sequencing data from TISCH2, we aimed to have a comprehensive understanding of the transcriptional landscape in melanoma patients undergoing immunotherapy. This integrated approach provides valuable insights into the molecular processes and potential biomarkers associated with response to anti-PD1/anti-CTLA4 therapy in melanoma. Our findings contribute to the growing body of knowledge in the field of tumor immunotherapy and may have implications for the development of personalized treatment strategies for melanoma patients.

Figure 1. Methodology diagram. We adopt a broad-to-specific approach. First, we determine the correlation of response and nonresponse with other factors before launching into analysis. We then input data into GSEA to determine upregulated pathways. scRNA-seq data was used to determine pathway enrichment occurrence in cells for the response group with another dataset of 31 patients. We used UMAP single cell profile analysis to determine pathway occurrence in cells and signature score comparison for key genes in pathways for our dataset, confirming our findings.

2.2. Preliminary correlation analysis

In the correlation analysis conducted, data from a total of 91 patients were utilized to explore the relationship between response and two variables: sex and age. By examining these factors, we aimed to uncover any potential associations between response and these demographic characteristics before continuing on our analysis

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2.3. Gene set enrichment analysis (GSEA)

In our study, we analyzed the data collected from 73 patients mentioned earlier. This data included both their clinical response information and other relevant factors. Our primary objective was to identify and investigate the pathways that were upregulated in these patients. To achieve this, we utilized Gene Set Enrichment Analysis (GSEA), a powerful tool commonly used in bioinformatics allowed us to gain insights into the molecular mechanisms and biological processes that may be associated with the observed clinical responses. Identifying these pathways is crucial for understanding the underlying biology and finding potential therapeutic targets.

2.4. Core gene expression and response analysis

We conducted an analysis of mRNA levels in key genes within pathways that were found to be enriched through Gene Set Enrichment Analysis (GSEA). We utilized bulk-RNA sequencing data from a cohort of 73 patients in order to investigate the correlation between these mRNA levels and the response to anti-PD1 therapy. By examining mRNA levels, we aimed to identify any patterns or associations that could provide insights into the effectiveness of this therapy. The goal of our analysis was to understand the potential role of these key genes in predicting response to anti-PD1 treatment.

2.5. UMAP single cell profile analysis

scRNA-seq allows for the examination of gene expression at a single cell level, providing a high-resolution view of cellular heterogeneity. We utilized scRNA-seq data obtained from 31 patients to generate UMAPs (Uniform Manifold Approximation and Projection) for the purpose of conducting single cell profile analysis. The objective was to establish a link between pathway upregulation and specific cell populations. By applying UMAPs to the scRNA-seq data, we visualized and clustered different cell populations based on their gene expression profiles. This approach enabled the identification of distinct cellular subtypes within the patient samples. We were also interested in investigating how pathway upregulation correlated with the identified cell populations. By analyzing gene expression data, we determined which pathways were significantly upregulated in specific cell types. This information could potentially shed light on the underlying molecular mechanisms driving cellular behavior and function in disease states.

2.6. Signature score analysis

In our study, we conducted an analysis of the signature score of genes at the single-cell level in immune cells found within melanoma tumors. Specifically, we aimed to compare the signature score of genes between tumors that responded to anti-PD1 treatment and those that did not. By examining the signature score, which is a measure of gene activity, we were able to gain insights into the immune response within these tumors and its correlation with treatment outcomes. Our analysis focused on immune cell as they play a crucial role in the tumor microenvironment and their response to treatment can greatly impact therapeutic efficacy.

3. Methodology

3.1. Immune checkpoint therapy improves overall survival

We collected clinical and tumor transcriptional sequencing data from 91 melanoma patients who were treated with anti-PD1/anti-CTLA4 therapy. 49 patients (~54%) had a clinically complete response (CR) or partial response (PR) to the treatment, while 42 patients (~47%) had no response to therapy. We found that anti-PD1/anti-CTLA4 responders (RP) had significantly better survival rates compared to non-responders (NR) ($p<0.0001$, Figure 2A). Several previous studies suggest that the immunotherapy response status may correlate with the patient's age and gender. However, we found that the anti-PD1/anti-CTLA4 response was not correlated to sex (two-tailed p-value > 0.05, Figure 2B) nor age (two-tailed p-value >0.05. Figure 2C) in these 91 melanoma patients. Thus, it is safe to directly compare the transcriptome profiles of response and nonresponse groups.

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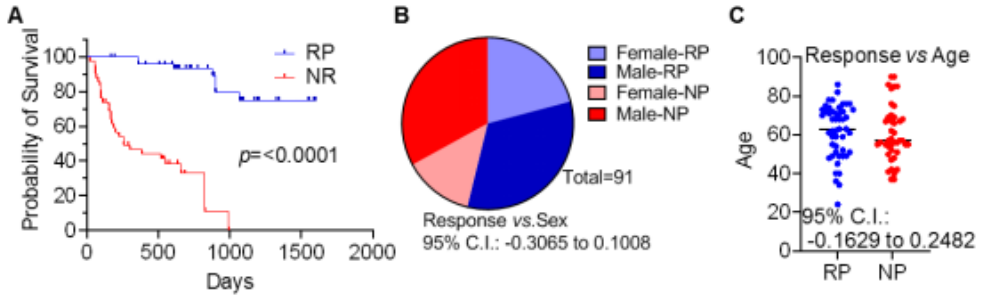

Figure 2. Immune checkpoint blockade therapy improves patient survival. (A) Survival curves of melanoma patients treated with anti-PD-1/anti-CTLA4 therapy, based on tumor response (RP, Blue) versus nonresponse (NR, Red). p values: log-rank test. (B) A pie chart illustrating the distribution of male and female melanoma patients treated with anti-PD-1/anti-CTLA4 therapy, based on their tumor response or lack thereof. (C) The age of melanoma patients treated with anti-PD-1/anti-CTLA4 therapy, based on their tumor response or lack thereof.

3.2. Immune-related pathways are associated with ICI response

We then evaluated whether transcriptomic features would differentiate between responding and non-responding tumors from 73 patients (responding, n=40; nonresponding, n=33). We found that genes that directly code the immune checkpoint molecules PD-1, PD-L1, PD-L2, and CTLA4, were differentially expressed between response versus non-response tumor groups (Figure 3). We also used Gene Set Enrichment Analysis (GSEA) to analyze the transcriptome data. We found that no hallmark gene sets were significantly upregulated in the nonresponse group (nominal p-value < 5% and FDR < 25% were considered as significant enrichment in this paper), while eight hallmark pathways were enriched in the response group (Figure 4). Interestingly, all of the enriched pathways are related to immune response, including the IFNγ response pathway, the IFNα response pathway, the allograft rejection pathway, the complement pathway, the IL6-JAK-STAT3 pathway, the IL2-STAT5 pathway, the TNFA-NFKB pathway, and the inflammatory response pathway.

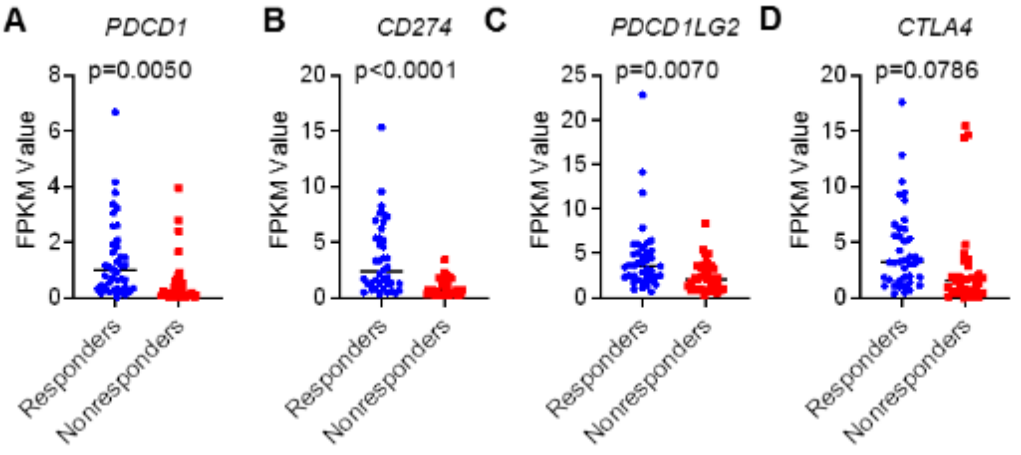

Figure 3. Genes in modulating immune checkpoint sensitivity were differentially expressed between responding versus non-responding tumors. (A-D) mRNA levels of PDCD1 (code PD1), CD274 (code PD-L1), PDCD1LG2 (code PD-L2), and CTLA4 in the responding versus non-responding pretreatment tumors.

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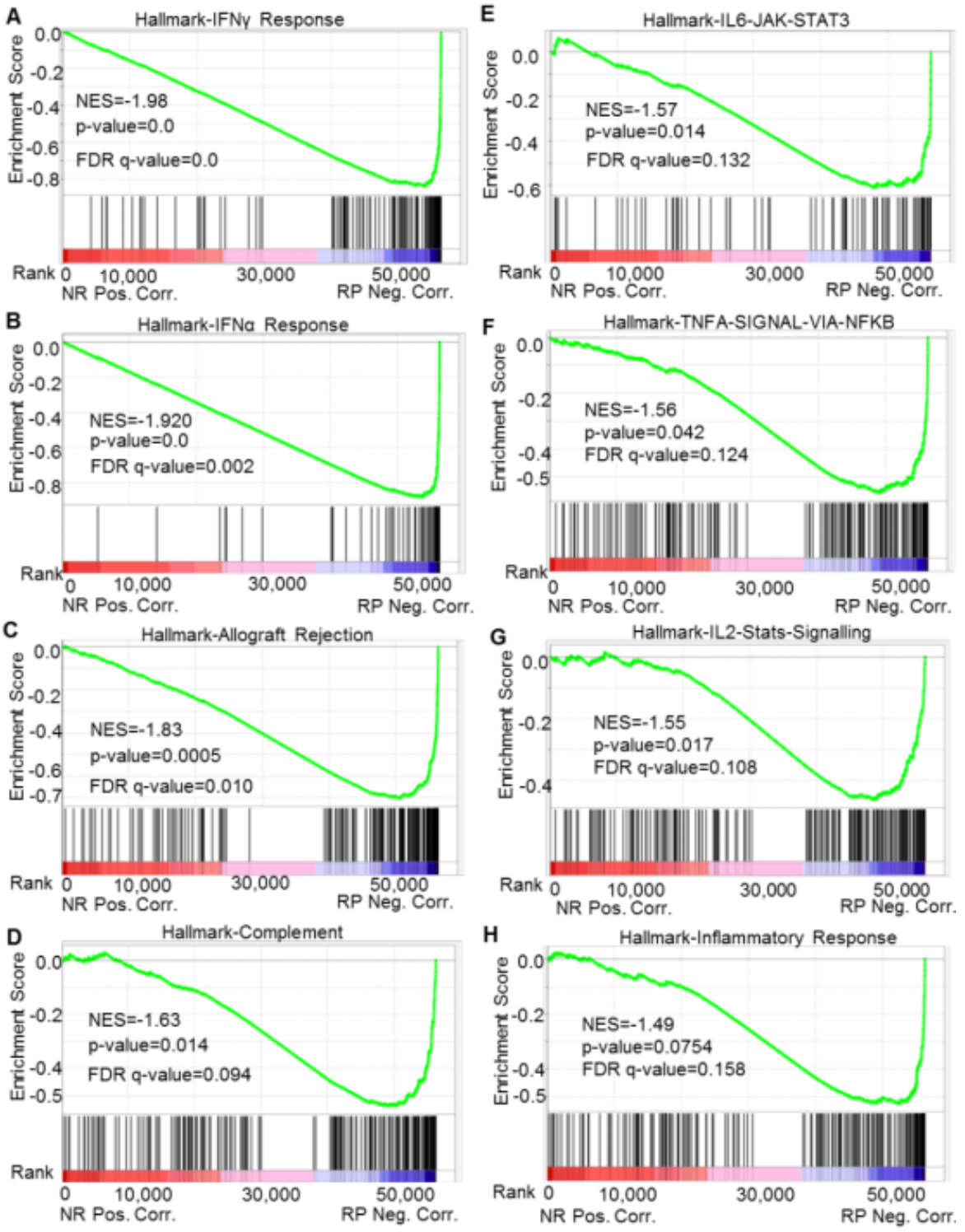

Figure 4. The immune-related pathways are upregulated in melanoma tumors that respond to anti-PD-1-/anti-CTLA4-treatment. (A-H) Gene set enrichment analysis (GSEA) results of bulk RNA-Seq data show signaling pathways enriched in melanoma tumors that respond to anti-PD-1-/anti-CTLA4-treatment.

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3.3. Antigen presentation and inflammatory tumor phenotypes are associated with innate anti-PD-1 response

We then evaluated the expression of core genes involved in the enriched pathways in the two groups. The differentially expressed genes (DEGs) suggest that the downregulated antigen presentation and inflammatory tumor phenotypes may be associated with innate anti-PD-1 resistance. DEGs that were expressed lower in nonresponding tumors included interferon α, β, γ and signature genes such as IFNG, JAK2, TYK2, STAT1, STAT2, IRF1, IRF2, IRF9, MX1, and OASL (Figure 5A), HLA class I signature genes such as TAP1, TAPBP, HLA-A, HLA-B, and HLA-C (Figure 5B), HLA class II signature genes such as HLA-DMA, HLA-DPA1, HLA-DPB1, HLA-DRA, and HLA-DRB1(Figure 5B), complement system signature genes such as C1QA, C1S, C1R and C3 (Figure 5A), and inflammatory response signature genes such as IL2RG, IL12RB1, IL15RA, IL17RA, CSF2RB, CCL5, CXCL9, CXCL10, and CXCL11 (Figure 6B).

Since bulk RNA-seq data contains transcriptional activity of both cancerous and non-cancerous cells, we then used single-cell RNA-seq data from 31 melanoma patients to map the cells in which the altered pathways were enriched compared to other cells. We found that the altered gene signature sets mainly enriched in the myeloid population (monocytes and macrophages) and T cells compared to other cell types (Figure 7). For instance, the IFNγ-response pathway, the IFNα-response pathway, the allograft rejection pathway, and the IL2-STAT5 pathway were highly enriched in the myeloid population (monocytes and macrophages) and T cells (Figure 7B-7D), while the TNFA-NFKB signal and inflammatory response pathways were highly enriched in the myeloid population (Figure 7F &7H). These results suggest that the myeloid and T populations are major players in effective response to anti-PD1/anti-CTLA4 therapy.

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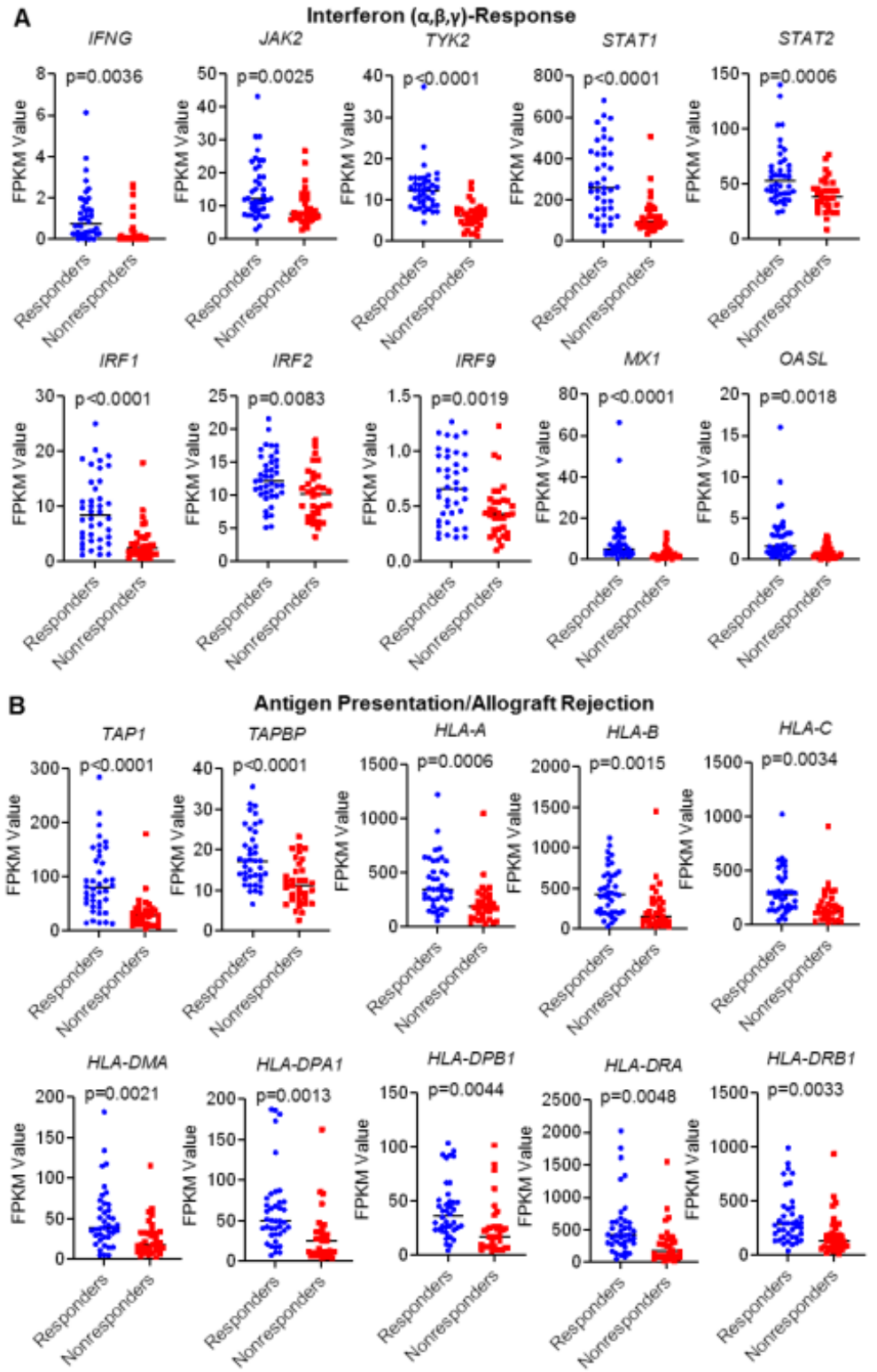

Figure 5. Downregulation of Interference Response and Antigen Presentation Pathway relates to Innate Resistance to anti-PD1-/anti-CTLA4 treatment. (A) mRNA levels of genes that control interference response which were differentially expressed between the responding versus non-responding pretreatment tumors; (B) mRNA levels of genes that control antigen presentation which were differentially expressed between the responding versus non-responding pretreatment tumors.

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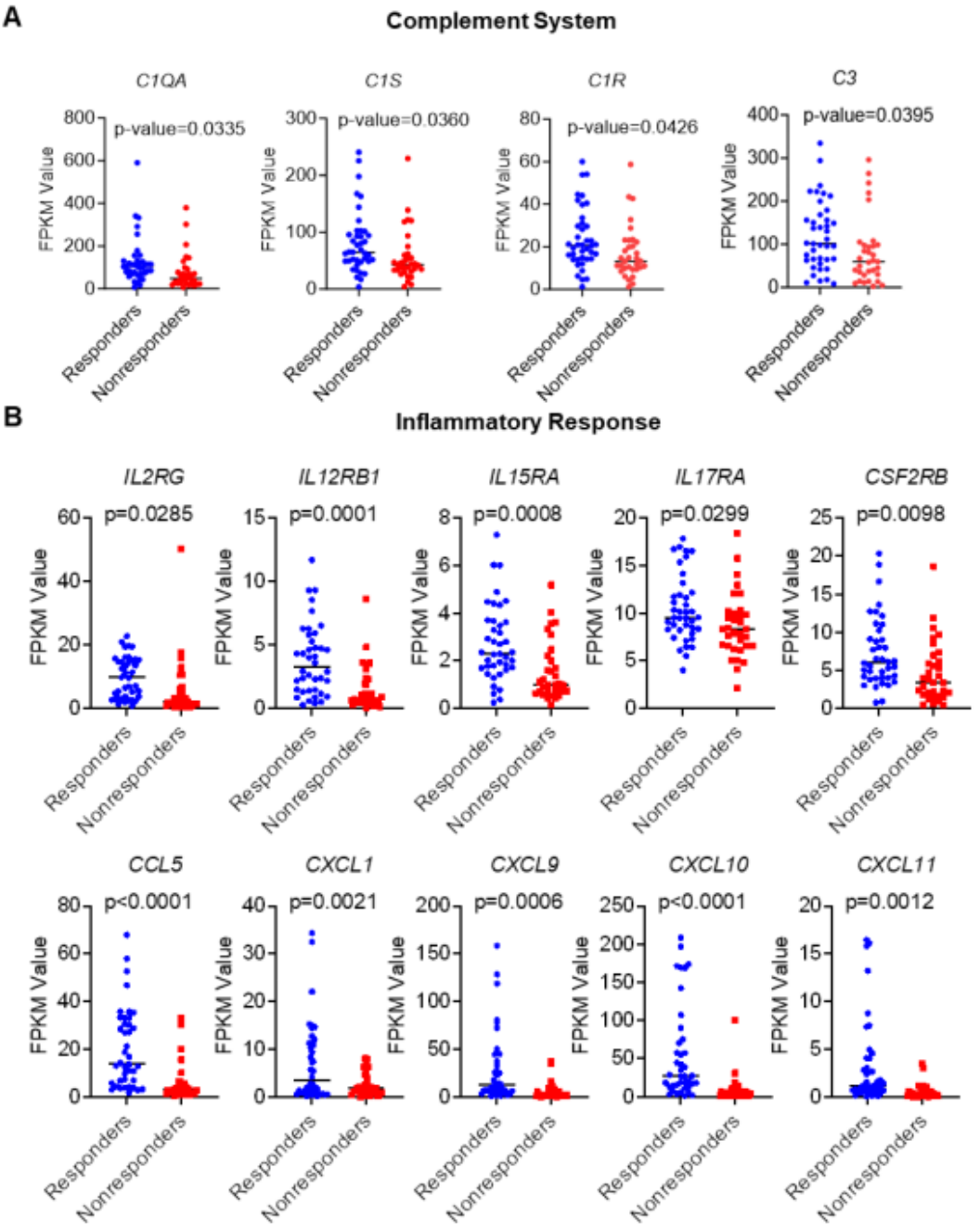

Figure 6. Upregulation of Inflammatory Response Pathway Correlates to Innate Resistance to anti-PD1-/anti-CTLA4 treatment. (A) mRNA levels of genes that control complement response which were differentially expressed between the responding versus non-responding pretreatment tumors; (B) mRNA levels of genes that control inflammatory response which were differentially expressed between the responding versus non-responding pretreatment tumors.

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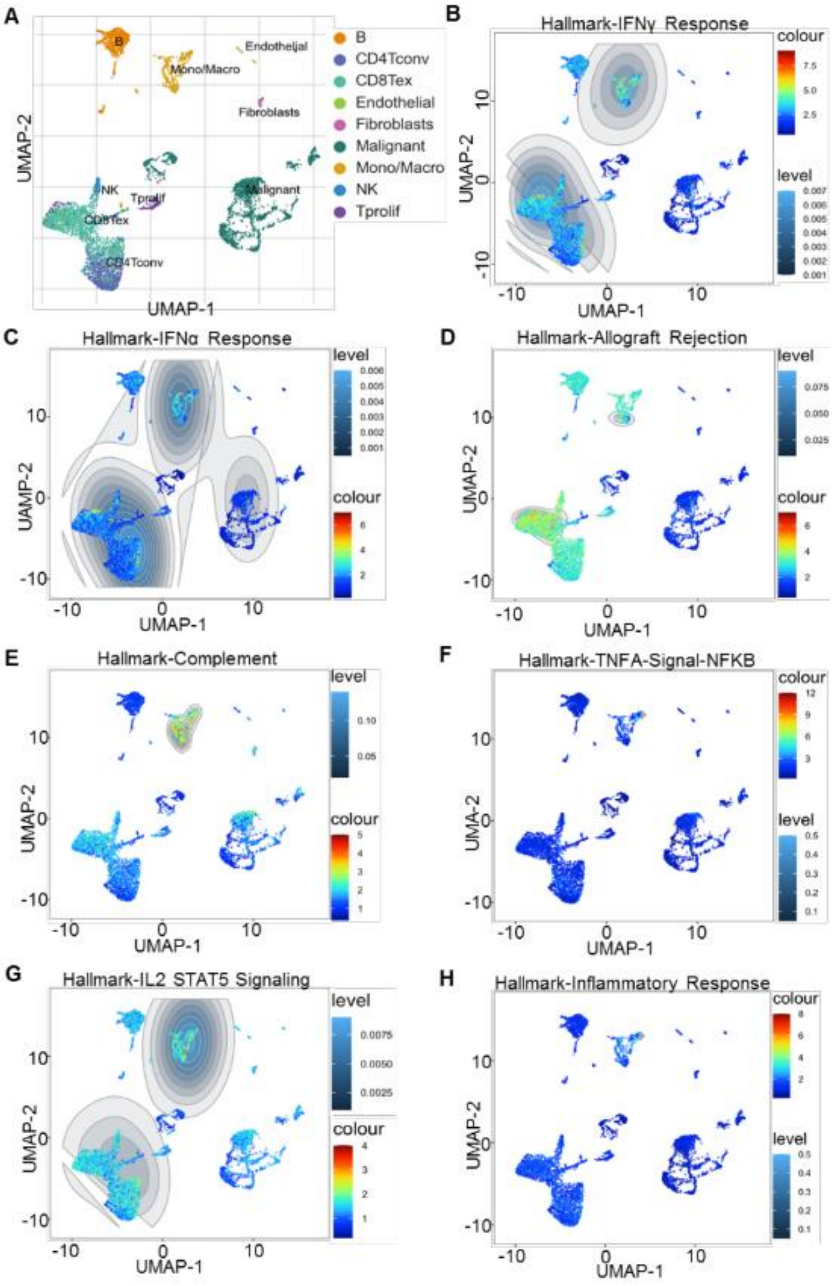

Figure 7. The Myeloid and T Cell Populations Contribute Transcriptomic Signatures of Innate Resistance to anti-PD1-/anti-CTLA4 treatment. (A) Distinct profiles of malignant and non-malignant cells in melanoma tumors. UMAP of single-cell profiles (dots) from malignant and non-malignant cells colored by post hoc annotation are shown. (B-H) UMAP of hallmark gene set signatures for IFNγ Response (B), Hallmark-IFNα Response (C), Hallmark-Allograft Rejection (D), Hallmark-Complement (E), Hallmark-TNFA-Signal-NFKB (F), Hallmark-IL2 STAT Signaling (G), and Hallmark-Inflammatory Response(H).

3.4. Immune cells contribute transcriptomic signatures of innate resistance

To further confirm whether the difference of the altered pathways based on bulk RNA-seq data was truly contributed by myeloid and T cells, we compared the signature score of gene sets at single-cell level from immune cells in melanoma tumors that respond or did not respond to anti-PD1 treatment. As shown in Figure 8, all enriched signaling pathways (in the responding tumor group) identified from bulk RNA-

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Seq data are down-regulated in myeloid and/or T cell populations, suggesting that the downregulated pathways in immune cells may contribute to innate resistance to anti-PD1/anti-CTLA4 treatment.

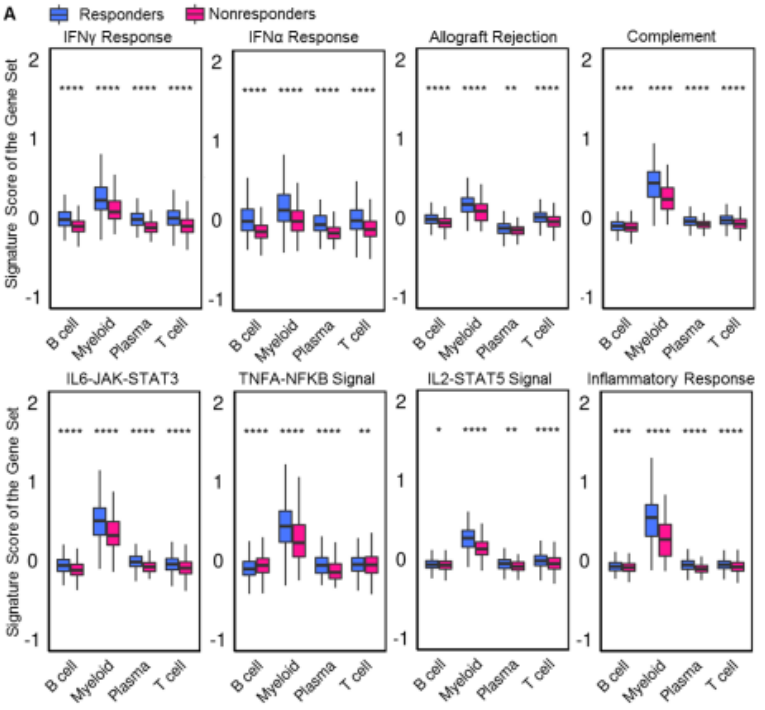

Figure 8. The immune-related pathways are downregulated in immune cells and contribute to transcriptomic signatures of innate resistance to anti-PD1-/anti-CTLA4 treatment. (A) Distribution of the signature scores of the indicated hallmark gene sets in immune cells from melanoma tumors that respond(blue) or did not respond (red) to anti-PD-1 treatment.

4. Discussion

We observed higher enrichment of multiple interferon (α and γ) signatures in the anti-PD1/anti-CTLA4-responsive group. The interferon γ signature was found to be highly expressed in the tumor from responding patients. We noted that multiple interferon response factors (IRF1, IRF2, and IRF9) are significantly upregulated in response groups compared to nonresponse groups (Figure 4A, p-value< 0.05). This finding matches the positive correlation of these genes with the same immune cell types in head and neck squamous carcinoma [11]. Coincidentally in head and neck squamous carcinoma, PDL1 had a significant correlation with IRF family genes [11]. Therefore, we hypothesize that higher expression of the IRF family gene may mean easier targets for anti-PDL1 therapy, explaining the positive correlation between IRF expression and anti-PDL1 therapy response in melanoma. This hypothesis is further verified by the literature-proven individual anti-cancer effects of IRF1 and IRF9 [12, 13].

IRF1 has been proven to correlate with PDL1 expression in lung cancer, which would provide more targets for anti-PD1/PDL1 therapy and provide a mechanism for better response [14]. A study shows that IRF1 could be used to predict anti-PD1 response, correlating with our findings that express a significantly high correlation of downregulation of IRF1 in nonresponse contrasting with higher expression levels of IRF1 in response ([15], Figure 4A, p value<0.001).

However, both IRF2 and IRF9 present special cases. IRF2 suppresses anti-tumor immune responses [13, 16]. On the other hand, sources confirm IRF2 expression is related to anti-PD1 immunotherapy sensitivity and increased immune response in colorectal cancer, probably because IRF2 represses PD-L1, helping anti-PD1 immunotherapy [17, 18]. Loss of IRF2 is correlated with less CD8+ cell activation and increased tumor evasion [17]. These findings support our data that IRF2 upregulation is correlated

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with patients responding to anti-PD1 therapy (Figure 4A, p value=0.0083). Nevertheless, an explanation is needed on the contradictory evidence regarding IRF2 and cancer regulation.

Similar data with IRF9 is also contradictory. IRF9 has been found to upregulate IL6 and aid in colorectal cancer growth, though in other cancers IRF9 has played a role in cell death and had anti-tumor effects. This diversity can be attributed to differences in vivo and in vitro conditions and different cancer cell types [19]. However, IRF9 may be part of a key pathway that increases PD-L1 expression in lung cancer cells, which matches with our results of high expression level correlation of IRF9 in melanoma cancer response group cells ([20], Figure 4A, p value=0.0019). This may mean that IRF9-expressing cells may indicate a better response to anti-PD1 therapy in melanoma, though this remains to be further verified.

The complement system is very complex and not well understood in melanoma. Researchers have found in clear-cell renal cell carcinoma that tumor-associated macrophages produced C1q while tumor cells produced C1R, C1S, and C3. This activates the classical complement pathway, which produces a pro-cancer effect. Other cancers and other models have shown anti-cancer effects of the complement system, pointing in the direction that the complement system's role may vary based on the type of cancer and the model used for testing. This matches with similar observations that the role of the complement system is highly dependent on the type of cancer and its effects may even vary depending on the model used [21]. Furthermore, a higher density of C1Q-producing tumor-associated macrophages was associated with increased expression of immune checkpoint molecules like PD-1 and PD-L1 and immunosuppressive environment [22]. This could mean both pro-cancer and anti-cancer effects, highlighting the complexity of the complement system in cancer. Even skin cell cutaneous melanoma (protective complement) shows different effects than uveal melanoma (aggressive complement) [21]. Therefore, we put our focus on melanoma-related studies. C1q-deficient mice with syngeneic B16 melanoma experience a slower rate of tumor growth and have an extended lifespan, suggesting that C1q plays tumor-suppressing effects in melanoma. The study also found that there was weak C3 expression in the sample as well, similar to our results of C3 (p value=0.0395) having a slightly weaker correlation of expression than C1QA (0.0335) in nonresponse patients (Figure 5A). This further indicates that the alternate pathway is also activated, not just the classical pathway [23]. Our results show that there is a correlation between low expression levels of C1QA, C1R, C1S, and C3 with nonresponse patients verifying the anti-tumor effects of the complement system in the mice melanoma tumor study (Figure 5A, p values < 0.05). A 2017 study analyzing both melanoma and colon cancer in mice found that anti-PD1/PDL1 was correlated with higher levels of C1q and C3b/iC3b/C3c (cleaved products of C3) within tumors [24]. C3 showed a higher response during anti-PD1 therapy compared to C1 (low anti-tumor immune response), leading to increased overall survival rates and progression-free survival [25]. This shows that C3 might be a good biomarker for enhancing anti-PD1 therapy, though this remains to be tested clinically. C1-specific functions remain to be confirmed in an anti-PD1 context.

5. Conclusion

In summary, clinical and tumor transcriptional sequencing data from large cohort of melanoma patients who received ICIs indicated that the IFNγ response pathway, the IFNα response pathway, the IL2-STAT5 pathway, the TNFA-NFKB pathway, and the inflammatory response pathway are significantly downregulated in non-responsive tumors pro-treatment. The single-cell transcriptome comparation of immune cells in melanoma that respond or did not respond to anti-PD1 treatment suggests that the difference of these gene sets might be contributed by myeloid and T cell populations. These findings suggest that the transcriptomic landscape (especially the identified gene sets) could be used as biomarkers to predict and preselect patients for ICIs. These analyses also indicate that attenuating the biological processes that underlie these pathways may improve ICI response in melanoma and other cancer types. but further clinical research is required to validate the impact of these gene sets.

References

[1] SArnold, M., Singh, D., Laversanne, M., Vignat, J., Vaccarella, S., Meheus, F., Cust, A. E., de Vries, E., Whiteman, D. C., & Bray, F. (2022). Global Burden of Cutaneous Melanoma in 2020 and Projections to 2040. JAMA Dermatology, 158(5), 495. https://doi.org/10.1001/jamadermatol.2022.0160

[2] Skin cancer statistics. (2023, March 22). World Cancer Research Fund International.

[3] Saginala, K., Barsouk, A., Aluru, J. S., Rawla, P., & Barsouk, A. (2021). Epidemiology of Melanoma. Medical Sciences, 9(4), 63. https://doi.org/10.3390/medsci9040063

[4] Huang, A. C., & Zappasodi, R. (2022). A decade of checkpoint blockade immunotherapy in melanoma: understanding the molecular basis for immune sensitivity and resistance. In Nature Immunology (Vol. 23, Issue 5, pp. 660–670). Nature Research. https://doi.org/10.1038/s41590-022-01141-1

[5] Hugo, W., Zaretsky, J. M., Sun, L., Song, C., Moreno, B. H., Hu-Lieskovan, S., Berent-Maoz, B., Pang, J., Chmielowski, B., Cherry, G., Seja, E., Lomeli, S., Kong, X., Kelley, M. C., Sosman, J. A., Johnson, D. B., Ribas, A., & Lo, R. S. (2016). Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. Cell, 165(1), 35–44. https://doi.org/10.1016/j.cell.2016.02.065

[6] Gellrich, F. F., Schmitz, M., Beissert, S., & Meier, F. (2020). Anti-PD-1 and novel combinations in the treatment of melanoma—an update. In Journal of Clinical Medicine (Vol. 9, Issue 1). MDPI. https://doi.org/10.3390/jcm9010223

[7] Lei, Q., Wang, D., Sun, K., Wang, L., & Zhang, Y. (2020). Resistance Mechanisms of Anti-PD1/PDL1 Therapy in Solid Tumors. In Frontiers in Cell and Developmental Biology (Vol. 8). Frontiers Media S.A. https://doi.org/10.3389/fcell.2020.00672

[8] Rizvi, N. A., Hellmann, M. D., Snyder, A., Kvistborg, P., Makarov, V., Havel, J. J., Lee, W., Yuan, J., Wong, P., Ho, T. S., Miller, M. L., Rekhtman, N., Moreira, A. L., Ibrahim, F., Bruggeman, C., Gasmi, B., Zappasodi, R., Maeda, Y., Sander, C., … Chan, T. A. (2015). Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. Science, 348(6230), 124–128. https://doi.org/10.1126/science.aaa1348

[9] Beasley, G. M., Brown, M. C., Farrow, N. E., Landa, K., Al-Rohil, R. N., Selim, M. A., Therien, A. D., Jung, S.-H., Gao, J., Boczkowski, D., Holl, E. K., Salama, A. K. S., Bigner, D. D., Gromeier, M., & Nair, S. K. (2022). Multimodality analysis confers a prognostic benefit of a T-cell infiltrated tumor microenvironment and peripheral immune status in patients with melanoma. Journal for ImmunoTherapy of Cancer, 10(9), e005052. https://doi.org/10.1136/jitc-2022-005052

[10] Lepletier, A., Madore, J., O'Donnell, J. S., Johnston, R. L., Li, X.-Y., McDonald, E., Ahern, E., Kuchel, A., Eastgate, M., Pearson, S.-A., Mallardo, D., Ascierto, P. A., Massi, D., Merelli, B., Mandala, M., Wilmott, J. S., Menzies, A. M., Leduc, C., Stagg, J., … Smyth, M. J. (2020). Tumor CD155 Expression Is Associated with Resistance to Anti-PD1 Immunotherapy in Metastatic Melanoma. Clinical Cancer Research, 26(14), 3671–3681. https://doi.org/10.1158/1078-0432.CCR-19-3925

[11] Liu, S., & Wang, Z. (2022). Interferon Regulatory Factor Family Genes: At the Crossroads between Immunity and Head and Neck Squamous Carcinoma. Disease Markers, 2022, 1–19. https://doi.org/10.1155/2022/2561673

[12] Qing, F., & Liu, Z. (2023). Interferon regulatory factor 7 in inflammation, cancer and infection. Frontiers in Immunology, 14, 1190841. https://doi.org/10.3389/fimmu.2023.1190841

[13] Savitsky, D., Tamura, T., Yanai, H., & Taniguchi, T. (2010). Regulation of immunity and oncogenesis by the IRF transcription factor family. Cancer Immunology, Immunotherapy, 59(4), 489–510. https://doi.org/10.1007/s00262-009-0804-6

[14] Chang, L.-C., Chen, T.-P., Kuo, W.-K., & Hua, C.-C. (2018). The Protein Expression of PDL1 Is Highly Correlated with Those of eIF2 α and ATF4 in Lung Cancer. Disease Markers, 2018, 1–9. https://doi.org/10.1155/2018/5068701

[15] Smithy, J. W., Moore, L. M., Pelekanou, V., Rehman, J., Gaule, P., Wong, P. F., Neumeister, V. M., Sznol, M., Kluger, H. M., & Rimm, D. L. (2017). Nuclear IRF-1 expression as a mechanism to assess "Capability" to express PD-L1 and response to PD-1 therapy in metastatic melanoma. Journal for ImmunoTherapy of Cancer, 5(1), 25. https://doi.org/10.1186/s40425-017-0229-2

[16] Lukhele, S., Rabbo, D. A., Guo, M., Shen, J., Elsaesser, H. J., Quevedo, R., Carew, M., Gadalla, R., Snell, L. M., Mahesh, L., Ciudad, M. T., Snow, B. E., You-Ten, A., Haight, J., Wakeham, A., Ohashi, P. S., Mak, T. W., Cui, W., McGaha, T. L., & Brooks, D. G. (2022). The transcription factor IRF2 drives interferon-mediated CD8+ T cell exhaustion to restrict anti-tumor immunity. Immunity, 55(12), 2369-2385.e10. https://doi.org/10.1016/ j.immuni. 2022.10.020

[17] Kriegsman, B. A., Vangala, P., Chen, B. J., Meraner, P., Brass, A. L., Garber, M., & Rock, K. L. (2019). Frequent Loss of IRF2 in Cancers Leads to Immune Evasion through Decreased MHC Class I Antigen Presentation and Increased PD-L1 Expression. The Journal of Immunology, 203(7), 1999–2010. https://doi.org/10.4049/jimmunol.1900475

[18] Liao, W., Overman, M. J., Boutin, A. T., Shang, X., Zhao, D., Dey, P., Li, J., Wang, G., Lan, Z., Li, J., Tang, M., Jiang, S., Ma, X., Chen, P., Katkhuda, R., Korphaisarn, K., Chakravarti, D., Chang, A., Spring, D. J., … DePinho, R. A. (2019). KRAS-IRF2 Axis Drives Immune Suppression and Immune Therapy Resistance in Colorectal Cancer. Cancer Cell, 35(4), 559-572.e7. https://doi.org/10.1016/j.ccell.2019.02.008

[19] Sharma, B. R., Karki, R., Sundaram, B., Wang, Y., Vogel, P., & Kanneganti, T.-D. (2022). The Transcription Factor IRF9 Promotes Colorectal Cancer via Modulating the IL-6/STAT3 Signaling Axis. Cancers, 14(4), 919. https://doi.org/10.3390/cancers14040919

[20] Morimoto, Y., Kishida, T., Kotani, S., Takayama, K., & Mazda, O. (2018). Interferon-β signal may up-regulate PD-L1 expression through IRF9-dependent and independent pathways in lung cancer cells. Biochemical and Biophysical Research Communications, 507(1–4), 330–336. https://doi.org/10.1016/j.bbrc.2018.11.035

[21] Revel, M., Daugan, M., Sautés-Fridman, C., Fridman, W., & Roumenina, L. (2020). Complement System: Promoter or Suppressor of Cancer Progression? Antibodies, 9(4), 57. https://doi.org/10.3390/antib9040057

[22] Roumenina, L. T., Daugan, M. V., Noé, R., Petitprez, F., Vano, Y. A., Sanchez-Salas, R., Becht, E., Meilleroux, J., Clec'h, B. Le, Giraldo, N. A., Merle, N. S., Sun, C.-M., Verkarre, V., Validire, P., Selves, J., Lacroix, L., Delfour, O., Vandenberghe, I., Thuilliez, C., … Fridman, W. H. (2019). Tumor Cells Hijack Macrophage-Produced Complement C1q to Promote Tumor Growth. Cancer Immunology Research, 7(7), 1091–1105. https://doi.org/10.1158/2326-6066.CIR-18-0891

[23] Bulla, R., Tripodo, C., Rami, D., Ling, G. S., Agostinis, C., Guarnotta, C., Zorzet, S., Durigutto, P., Botto, M., & Tedesco, F. (2016). C1q acts in the tumour microenvironment as a cancer-promoting factor independently of complement activation. Nature Communications, 7(1), 10346. https://doi.org/10.1038/ncomms10346

[24] Zha, H., Han, X., Zhu, Y., Yang, F., Li, Y., Li, Q., Guo, B., & Zhu, B. (2017). Blocking C5aR signaling promotes the anti-tumor efficacy of PD-1/PD-L1 blockade. OncoImmunology, 6(10), e1349587. https://doi.org/10.1080/2162402X.2017.1349587

[25] Subramanian, M., Kabir, A. U., Barisas, D., Krchma, K., & Choi, K. (2023). Conserved angio-immune subtypes of the tumor microenvironment predict response to immune checkpoint blockade therapy. Cell Reports Medicine, 4(1), 100896. https://doi.org/10.1016/j.xcrm.2022.100896