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IRF8 Deleterious Single Nucleotide Variations Are Associated with Unfavorable Survival of Patients with Skin Cutaneous Melanoma

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Abstract: Interferon regulatory factor 8 (*IRF8*) is a transcription factor involved in the differentiation of myeloid progenitors. In the current study, we aimed to explore the correlation between *IRF8* expression and immune cell infiltration in skin cutaneous melanoma, the single nucleotide variants (SNVs) of this gene, and their association with survival outcomes. Analyses are performed using data from The Cancer Genome Atlas (TCGA)-Skin Cutaneous Melanoma (SKCM), with the platform of Gene Set Cancer Analysis (GSCA). *IRF8* expression has an infiltration score of 0.7, suggesting a strong correlation with immune cell infiltration. *IRF8* expression had strong positive correlations (Pearson's $r \geq -0.6$) with the infiltration of CD4+ T, CD8+ T, Central memory T, Cytotoxic T, T follicular helper, Th1 and induced Tregs cells but presented a strong negative correlation (Pearson's $r \leq -0.6$) with the infiltration of Neutrophil cells. 8 out of 468 cases (1.71%) in TCGA-SKCM have deleterious SNVs. The *IRF8* mutant group harboring these mutations had significantly shorter progression-free survival ($P = 0.03$), disease-specific survival ($P = 0.0065$), and overall survival ($P = 0.039$) compared to the group with wild-type *IRF8*. In summary, *IRF8* expression might serve as an immune cell infiltration marker in skin cutaneous melanoma. *IRF8* deleterious SNVs are associated with significantly worse prognosis. In the future, it is meaningful to validate the link between these mutations and immunotherapy responses.

Keywords: *IRF8*, Skin Cutaneous Melanoma, SNVs, Immune Cell Infiltration, Prognosis

1. Introduction

Skin cutaneous melanoma is a highly malignant skin cancer [1]. For patients with primary and early stages of melanoma, surgical resection usually brings a favorable prognosis [2]. For patients with local control in oligometastatic disease, metastasectomy is also a potential strategy if complete macroscopic resection can be done [3]. However, for cases with advanced diseases, surgery is not sufficient.

In the past decade, immunotherapy demonstrated promising therapeutic efficiency for patients with advanced-stage melanoma [4]. The clinical utilization of immune checkpoint inhibitors, typically the monoclonal antibodies blocking programmed death receptor-1 (PD-1) and its associated ligand 1 (PD-L1) have drastically improved their overall survival (OS) [4, 5]. Nivolumab (anti-PD-1)/ipilimumab (anti-CTLA-4) combination can elevate five-year survival of metastatic melanoma from about 35% to 50% [6]. Although melanoma is one of the most sensitive tumors to immunotherapy, the therapeutic responses and duration largely depend on the type and abundance of immune cells in the tumor microenvironment [7, 8].

Interferon regulatory factor 8 (*IRF8*) is an IFN-γ-inducible transcription factor and acts as a tumor suppressor gene in multiple cancers [9]. Melanoma-bearing IRF-$8^{-/-}$ mice has significantly reduced immune cell infiltration [10]. *IRF8* complexes can drive the IL-9-producing T-helper cells (Th9) development and boost Th9 responses in cancer therapy [11]. Therefore, its expression might be a marker of immunotherapeutic response. However, its expression is progressively lost during melanoma growth [10]. Low *IRF8* expression is associated with significantly reduced survival of

patients with skin cutaneous melanoma [12].

Somatic mutations of the *IRF8* gene could impair *IRF8* transcriptional activity and lead to human dendritic-cell immunodeficiency [13]. These findings imply that *IRF8* mutations might interrupt its tumor-suppressive effects and affect patient survival. In the current study, we explored the correlation between *IRF8* expression and immune cell infiltration in skin cutaneous melanoma. Then, we analyzed the deleterious single nucleotide variants (SNVs) of the *IRF8* gene and assessed their association with survival outcomes.

2. Materials and Methods

2.1. Data Extraction and Bioinformatic Analysis

The RNA sequencing data, including gene expression and SNVs of the *IRF8* gene among the patients with skin cutaneous melanoma were obtained from The Cancer Genome Atlas (TCGA)-Skin Cutaneous Melanoma (SKCM) [14].

Bioinformatic analysis was performed to check the SNV types of *IRF8* and their association with survival among the patients, using the Gene Set Cancer Analysis (GSCA), which is an integrated web server for cancer data analysis [15]. The differences in progression-free survival (PFS), disease-specific survival (DSS) and overall survival (PFS) were compared between the groups with *IRF8* SNVs or without *IRF8* SNVs (wild-type).

2.2. Statistical Analysis

The correlations between *IRF8* expression and the infiltration of immune cells were assessed by calculating Pearson's correlation coefficients. The log-rank test was utilized to assess the survival differences. $P < 0.05$ were considered significant.

3. Results

3.1. IRF8 Expression Is Strongly Correlated with the Infiltration of Multiple Types of Immune Cells in Skin Cutaneous Melanoma

Using the immune cell infiltration data in GSCA [15], we assessed the correlation between *IRF8* expression and the infiltration of multiple types of immune cells in skin cutaneous melanoma (Figure 1). The detailed correlation coefficients are summarized in Table 1. *IRF8* expression has an infiltration score of 0.7, suggesting a strong correlation with immune cell infiltration (Figure 1). By using |Pearson's r|≥0.6 as the cutoff, we identified the key immune cell types strongly correlated with *IRF8* expression. Typically, the infiltration of CD4+ T, CD8+ T, Central memory T, Cytotoxic T, T follicular helper (Tfh), Th1 and induced Tregs (iTreg) cells showed strong positive correlations (Pearson's r ≥ -0.6) with *IRF8* expression (Table 1). In comparison, the infiltration of Neutrophil cells showed a strong negative correlation (Pearson's r ≤ -0.6) with *IRF8* expression (Table 1).

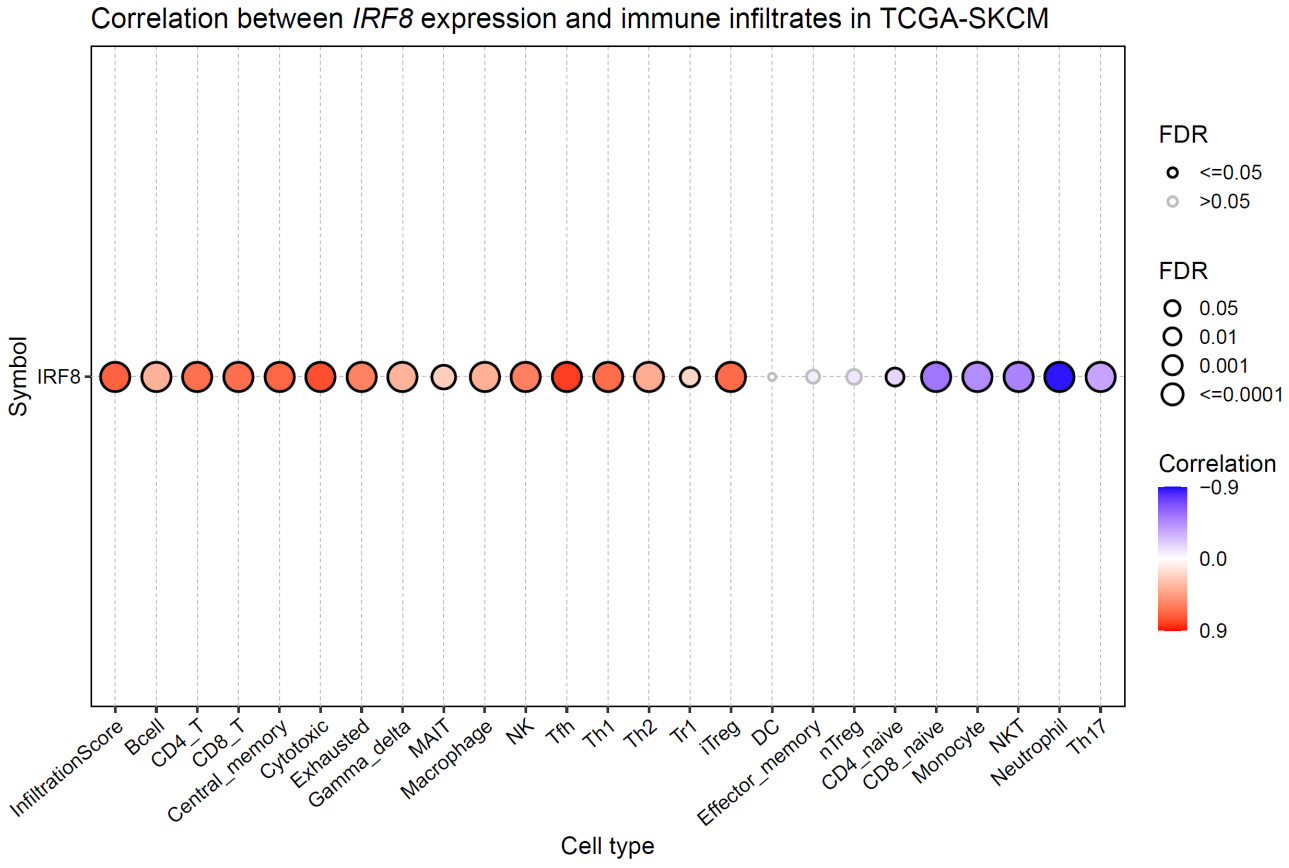

Figure 1. *A bubble plot showing the correlation between IRF8 expression and immune cell infiltration in TCGA-SKCM.*

Table 1. *A summary table showing the correlation coefficients between IRF8 expression and immune cell infiltration in TCGA-SKCM.*

Cancer type	Gene symbol	Cell type	Pearson's r	*P* value
TCGA-SKCM	*IRF8*	B cell	0.363	<0.001
		CD4 T	0.643	<0.001
		CD4 naive	-0.156	0.001
		CD8 T	0.651	<0.001
		CD8 naive	-0.537	<0.001
		Central memory T	0.683	<0.001
		Cytotoxic T	0.760	<0.001
		DC	0.001	0.980
		Effector memory	-0.074	0.106
		Exhausted	0.569	<0.001
		Gamma delta T	0.355	<0.001
		MAIT	0.233	<0.001
		Macrophage	0.368	<0.001
		Monocyte	-0.447	<0.001
		NK	0.584	<0.001
		NKT	-0.486	<0.001
		Neutrophil	-0.868	<0.001
		Tfh	0.812	<0.001
		Th1	0.656	<0.001
		Th17	-0.356	<0.001
		Th2	0.387	<0.001
		Tr1	0.190	<0.001
		iTreg	0.663	<0.001
		nTreg	-0.096	0.037

3.2. Missense Mutation Is the Dominant Deleterious SNVs of the IRF8 Gene in Skin Cutaneous Melanoma

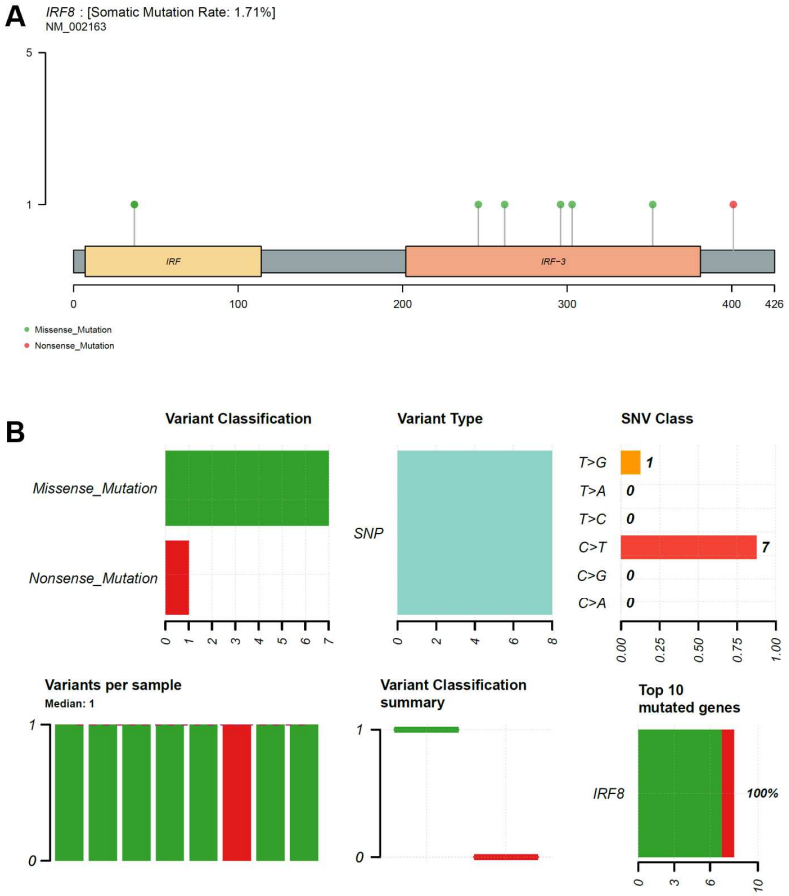

Figure 2. *The type of IRF8 deleterious SNVs in patients with skin cutaneous melanoma.*

A. A representative *IRF8* gene structure with the marked position of deleterious SNVs in TCGA-SKCM. B. An analysis of the composition of deleterious SNVs (missense and nonsense mutations). Data were obtained from TCGA-SKCM, via the access provided by Gene Set Cancer Analysis (GSCA): http://bioinfo.life.hust.edu.cn/GSCA/#/.

Using data from TCGA-SKCM, we checked the SNVs of *IRF8*. Our analysis confirmed 8 out of 468 cases (1.71%) have deleterious SNVs (Figure 2A). The positions of the SNVs were marked in gene structure in Figure 2A. The 8 SNVs include 7 missense mutations and 1 nonsense mutation (Figure 2B).

3.3. IRF8 Deleterious SNVs Are Associated with Unfavorable Survival Among Patients with Skin Cutaneous Melanoma

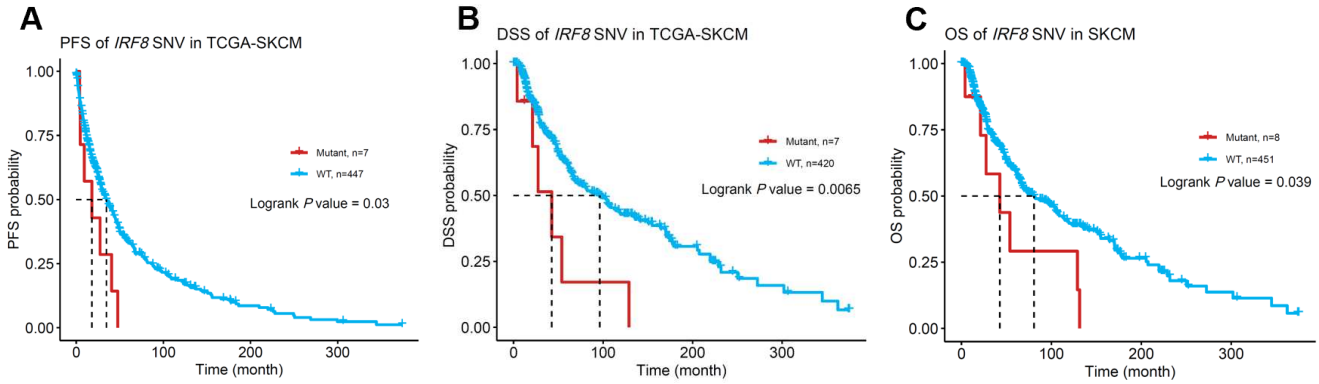

Figure 3. *Survival analysis of IRF8 SNVs and survival of patients with skin cutaneous melanoma.*

A-C. Kaplan-Meier survival analysis of PFS (A), DSS (B), and OS (C) in patients with skin cutaneous melanoma. The difference in survival was compared between the groups with deleterious *IRF8* SNVs (mutant, red line) and wild-type (WT, blue line) *IRF8*. Log-rank test was performed to assess the survival difference.

Since *IRF8* has been confirmed as an important controller of melanoma progression [10], we checked whether the deleterious SNVs are associated with survival outcomes. The differences in PFS, DSS, and OS were compared. Log-rank test indicated that the *IRF8* mutant group (with missense and nonsense mutations) had significantly shorter PFS (P=0.03) (Figure 3A), DSS (P=0.0065) (Figure 3B), and OS (P=0.039) (Figure 3C) compared to the group with wild-type (WT) *IRF8*.

4. Discussion

Based on the data from TCGA-SKCM (a large database with over 400 cases), we assess the correlation between *IRF8* expression and the infiltration of multiple types of immune cells in skin cutaneous melanoma. We observed that *IRF8* expression showed strong positive correlations with the infiltration of CD4+ T, CD8+ T, Central memory T, Cytotoxic T, Tfh, Th1, and iTreg cells and a strong negative correlation with Neutrophil cells. The response to PD-1 blockade therapy is associated with the recruitment of CD4+ and CD8+ T cells and the activation of central/effector memory T cells, Tfh, and Th1 cells [16, 17]. However, for patients with advanced cutaneous melanoma who received PD-1 blockade therapy, high infiltration of iTreg can predict poor therapeutic response to the inhibitor and is linked with an unfavorable prognosis [18]. *IRF8* can selectively induce and sustain the production of soluble factors via binding to different gene promoters. These factors are cooperatively involved in immunosurveillance and immune escape [9]. Although these findings suggest that *IRF8* expression might serve as an immune cell infiltration marker and might be linked with a favorable prognosis in skin cutaneous melanoma, the dark side of its influence on immune escape should be further explored in the future.

Missense and nonsense mutations of *IRF8* gene might generate a protein that does not function properly or is even non-functional [19, 20]. One previous study confirmed that *IRF8* K108E and T80A mutations could impair its transcriptional activity by reducing its binding with targeting DNA sequence, thereby hampering the differentiation of mononuclear phagocytes [13]. Considering the critical regulative effects of this protein on immune cell infiltration, we further checked its deleterious SNVs in skin cutaneous melanoma. Our findings indicated that although the deleterious SNVs are not common (8 out of 468 cases, 1.71%), they are associated with significantly worse survival, including PFS, DSS, and OS. It is reasonable to infer that the tumors harboring these mutations have dysregulated immune cell infiltration. These alterations might contribute to poor responses to immunotherapy, leading to a poor prognosis. However, this hypothesis needs to be validated in future clinical studies. For patients with the mutations, additional therapeutic surveillance or supportive care might be considered.

5. Conclusion

IRF8 expression is correlated with the infiltration of multiple types of immune cells in skin cutaneous melanoma and might serve as an immune cell infiltration marker. *IRF8* deleterious SNVs (missense and nonsense mutations) are associated with significantly worse PFS, DSS, and OS. In the future, it is meaningful to validate the link between these mutations and immunotherapy responses.

Conflict of Interest

All the authors do not have any possible conflicts of interest.

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