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Research article

The roles of genetic mutation and cytokines/chemokines in immune response and their association with uveal melanoma patient outcome

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ABSTRACT

The impact of tumor mutations and the interplay of cytokines and chemokines on the immune response and clinical outcomes in uveal melanoma (UM) warrants further exploration. In our study, we delved into the correlation between genetic alterations and survival rates in a cohort of 188 UM patients, utilizing data from cBioPortal. We assessed the composition of immune cell populations within 80 UM tumors by examining RNA sequence-based gene expression data from The Cancer Genome Atlas (TCGA). Furthermore, we scrutinized the relationship between genetic mutations and the expression of cytokines and chemokines, as well as their influence on various immune cell subsets. Our investigation revealed a significant association between the presence of mutated GNAQ or SF3B1 genes and improved progression-free survival (PFS), disease-specific survival (DSS), and overall survival (OS) when compared to patients with non-mutated counterparts. In contrast, the presence of immune response gene mutations was associated with a detrimental effect on PFS, DSS, and OS. We also observed that the expression levels of cytokines and chemokines were positively linked to the infiltration of immune killer cells and inversely related to the populations of B cells and dendritic cells. Elevated expression levels of PDCD1, TNF, IL6, CXCL9, and CXCL10 were found to be correlated with reduced OS. Intriguingly, an increase in $CD8^+$ T cell populations was associated with a poorer OS, a finding that warrants further investigation. These findings underscore the potential utility of cytokines/chemokines expression levels, immune cell subsets, and mutation status as critical biomarkers for the selection of patients who are most likely to benefit from immunotherapeutic interventions. Our research provides valuable insights that could guide the development of more targeted and effective treatment strategies for UM patients.

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1. Introductions

Uveal melanoma (UM) is a rare but highly aggressive form of cancer that predominantly affects the Caucasian population, with a particularly grim prognosis for those in advanced stages. A multitude of variables contribute to the development and progression of UM. Intrinsic host factors such as light iris color, fair complexion, limited tanning capacity [1], the presence of oculodermal melanocytes [2], dysplastic nevi [3], and genetic mutations [4] are known to play a significant role in the oncogenesis of UM. Additionally, external environmental factors, including exposure to sunlight [5,6], cannot be overlooked in contributing to the aggressive nature of this disease. Clinical features of UM, including an increased age at the time of diagnosis [7], larger tumor dimensions [8], and a diagnosis at a later stage of the disease [7], are often correlated with a less favorable prognosis. The tumor microenvironment (TME) is a complex ecosystem composed of various cell types, including tumor cells, stromal cells, immune cells, and endothelial cells. Within this milieu, tumor-infiltrating lymphocytes (TILs) are pivotal in shaping the immune response and influencing the efficacy of treatments for solid tumors [9–12]. Advancements in RNA sequencing (RNA-seq) have enabled the deconvolution of the TME, allowing for a detailed evaluation of the components and functional roles of different immune cell subsets [13–16] and this cutting-edge technology provides valuable insights into the cellular dynamics within the TME and is instrumental in discerning the contributions of these cells to disease progression.

Genetics has a key role in predisposition to UM and in its initiation and progression [17].The top 5 genes associated with Uveal Melanoma (UM) [18,19]based on the provided literature are:

BAP1 (BRCA1-associated protein 1): This is a tumor suppressor gene, and its genetic mutation leading to a loss of gene function is common in UM. The presence of BAP1 mutations is associated with a worse prognosis in patients with UM [20].

EIF1AX (Eukaryotic translation initiation factor 1A, X-linked): Mutations in this gene have been linked to the production of neoantigens that can be recognized by the host's $CD8^+$ T cells as extrinsic antigens. These neoantigens are correlated with the long latency and rare metastatic outgrowth of class 1 UMs [21].

SF3B1 (Splicing factor 3b, subunit 1): Mutations in SF3B1 are also associated with the production of neoantigens that can be recognized by the immune system, similar to EIF1AX mutations. These mutations have been correlated with the prognosis of UM [21].

GNAQ (Guanine nucleotide-binding protein Q): Mutations in GNAQ have been found to be associated with better progression-free survival (PFS), disease-specific survival (DSS), and overall survival (OS) in UM patients [22].

GNA11 (Guanine nucleotide-binding protein 11): GNA11 plays a significant role in the development of uveal melanoma, as mutations in this gene are found in a substantial proportion of these tumors, with such mutations leading to the activation of the mitogen-activated protein kinase pathway and contributing to tumor growth and metastasis [23].

These genes play crucial roles in the predisposition to UM, its initiation, progression, and response to immunotherapy. The genetic mutations in these genes can lead to the production of neoantigens that can be targeted by the immune system, potentially serving as biomarkers for immunotherapy responsiveness [19,24]. However, the presence of lymphocyte infiltrates in UM, which is associated with mutations in these genes, paradoxically predicts a poor prognosis, indicating a complex relationship between the tumor's genetic makeup and the immune response in UM [25]. The low somatic mutation frequency in UM compared to cutaneous melanoma suggests a higher resistance to immune therapy in UM [23]. Understanding the role of these genes and their interactions with the immune system is essential for exploring the immunobiology of UM and identifying novel therapeutic targets [26].

Cytokines and chemokines are pivotal regulatory molecules that orchestrate the intricate crosstalk between immune and non-immune cells, playing a crucial role in the mutated genes influence disease progression through the immune response to cancer. These molecules are instrumental in the recognition of tumor neoantigens by the human immune system, which is essential for the enrichment of tumor-infiltrating lymphocytes (TILs) and the upregulation of type II interferon (IFN-γ or IFNG)-related genes, such as PD-L1 and CTLA-4. A Clinical research has demonstrated a positive correlation between the expression of tumor interferon IFNG and improved clinical outcomes in various cancer types [27]. Furthermore, the transforming growth factor-beta (TGF-β) has been shown to modulate UM cell migration and invasion at metastatic sites [28]. In the process of UM metastasis and progression, IL-6 has been implicated in the promotion of UM cell invasion and migration. Its effects include the suppression of cell-cell adhesion and the enhancement of focal adhesion. The IL-6/STAT3/JunB axis is particularly noteworthy for its role in promoting UM aggressiveness through the process of epithelial-mesenchymal transition (EMT) in response to IL-6 stimulation [29]. Furthermore, research by Nagarkatti-Gude et al. has revealed a correlation between elevated IL-6 levels and increased tumor prominence, as well as the infiltration of macrophages and regulatory T cells (Tregs) within the UM tumor microenvironment [30].

Chemokines are essential guides in the immune system's navigation, directing the migration of immune cells towards the tumor microenvironment. In the case of cutaneous melanoma, specific chemokines, such as CXCL9 and CXCL10, have been noted to be upregulated in tumors that harbor an abundance of T cells. This preferential expression is indicative of an active immune response within the tumor milieu [31]. Moreover, elevated levels of CCL5, CXCL9, and CXCL10 have been associated with improved clinical outcomes in melanoma patients, particularly those who have been treated with a melanoma vaccine [32]. However, in the context of uveal melanoma (UM), a distinct picture emerges. The expression patterns of CX3CL1, CXCL9, and CXCL10, which are also found in higher concentrations in the vitreous fluid associated with UM, do not show a clear distinction between tumors that do and do not have tumor-infiltrating lymphocytes [32]. This observation underscores the complexity of the immune response in UM and highlights the need for a nuanced understanding of the chemokine and cytokine profiles in this specific type of cancer.

In our current study, we initially investigated the correlation between genetic mutations and the progression of uveal melanoma (UM).We then applied deconvolution algorithms—Tumor Immune Estimation Resource (TIMER) 2.0 [16], Estimating the Proportions of Immune and Cancer cells (EPIC) [14] and Cell type Identification By Estimating Relative Subsets Of known RNA Transcripts-Absolute (CIBERSORT-ABS) [15], to evaluate cellular fractions from UM RNA-seq data in The Cancer Genome Atlas

(TCGA); Finally, we examined the associations between mutations and the expression of cytokines/chemokines with immune cell populations and disease outcomes.

2. Materials and methods

2.1. UM dataset patients

We collected demographic information, genetic mutation data, clinical variables, and follow-up details for 188 uveal melanoma (UM) patients from cBioPortal (https://www.cbioportal.org/). Prognostic risk factors included the 2009 American Joint Committee on Cancer (AJCC) stage, tumor mutational load, the primary site of the tumor, and its location. Complete clinical data were accessible for all 188 patients.

Utilizing the UM dataset from TCGA (https://portal.gdc.cancer.gov/projects/TCGA-UVM), we evaluated the immune cell composition and identified key biomarkers associated with immune response and patient outcomes in a cohort of 80 UM patients. The RNA-seq data was generated using the IlluminaHiSeq_RNASeqV2 platform, which facilitated the quantification of mRNA gene expression.

2.2. Immune cell fraction of UM tumors& deconvolution analysis

We performed a detailed analysis of RNA-sequencing data from 80 uveal melanoma (UM) patients within The Cancer Genome Atlas (TCGA) to evaluate the immune cell composition. Utilizing the TIMER2.0 algorithm, we deconvoluted the transcriptomic profiles to estimate the relative abundance of six primary immune cell subsets: B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, dendritic cells, and macrophages. TIMER2.0 employs a reference gene expression matrix that accounts for tissue-specific effects, thereby enhancing the accuracy of deconvolution, and requires specification of cancer type for custom data inputs [16]. Given the typically minor proportions of natural killer (NK) cells and regulatory T cells (Tregs) within tumors, we complemented TIMER2.0 with EPIC and CIBERSORT-ABS, respectively, to ensure these cell populations were accurately represented [14,15]. Both EPIC and CIBERSORT-ABS use curated gene expression reference matrices and heuristic algorithms to determine the cellular composition of the samples.

To ensure the reliability of our deconvolution analysis, we meticulously standardized the RNA-seq data through median-centering and unit-normalization of the variables. This preprocessing step was essential for aligning the data on a uniform scale, thereby facilitating a consistent and comparable interpretation of the immune cell composition across the TCGA UM tumor samples. The integration of these methodologies allowed us to comprehensively quantify and analyze the immune cell subsets present in the UM tumor microenvironment, providing valuable insights into the complex interplay between tumor cells and the immune system in UM.

2.3. Immune response related to UM patient survival

We conducted an analysis using TIMER2.0 to assess the immune cell composition in 80 uveal melanoma (UM) patients with RNA-sequencing data available in The Cancer Genome Atlas (TCGA) [16]. The TIMER2.0 method utilizes a reference gene expression matrix to calculate the relative proportions of six key immune cell subsets, taking into account tissue-specific effects. For enhanced accuracy, this method requires the input of cancer type information when processing custom data. Additionally, we utilized EPIC to estimate the presence of natural killer (NK) cells and CIBERSORT-ABS to quantify regulatory T cells (Tregs), as these cell types are typically found in smaller quantities within tumor samples.

These tools rely on an internal reference gene expression matrix to determine the cellular composition of the tumor samples. Through these methodologies, we were able to estimate the immune cell subsets within the TCGA UM tumor dataset for this study.

2.4. Statistical analysis

Initially, We identified the five most commonly mutated genes in uveal melanoma (UM) — GNAQ, GNA11, BAP1, SF3B1, and EIF1AX — across 188 tumor samples and assessed their impact on patient outcomes, including disease-free survival (DFS), progression-free survival (PFS), disease-specific survival (DSS), and overall survival (OS) [33]. DFS is a composite endpoint typically defined as time until local recurrence, contralateral recurrence (and/or new primary breast cancers), distant disease, secondary cancers or death from any cause. Progression free survival (PFS) is defined as the time from randomization until first evidence of disease progression or death. DSS was defined as the time from the date of diagnosis of the primary tumor to the date of disease-specific death or follow up for patients remaining alive [34]. OS was defined as the time between diagnosis and death from any cause. Patients who had not relapsed/died or died were censored at the date of the last follow-up visit. Within 80 TCGA UM tumor samples, we evaluated immune cell subsets and compared immune infiltration between mutated and wild-type samples using the Wilcoxon test. We also examined selected cytokines and chemokines related to the tumor immune response or clinical outcomes, exploring their correlation with T-cell infiltration and survival [27,31,35–38]. Spearman correlation was used to quantify the relationship between chemokine/cytokine gene expression and the immune response, enhancing our understanding of the immune dynamics in UM [39].

Utilizing TCGA data, we conducted a series of analyses focused on patients with available survival data. We initiated with a univariate survival analysis to assess the impact of the $CD8^+$ T cell population on overall survival (OS). Subsequently, we performed a multivariable analysis, controlling for age, sex, disease stage, and tumor purity, which is the proportion of cancer cells within a tumor tissue sample. All data analyses were executed using R language [40] and the SAS Enterprise Guide 4.3 software program (SAS Institute

Inc.). Statistical significance was defined by a two-sided P value of less than 0.05 [41]. To address the issue of multiple comparisons, we applied the Bonferroni adjustment when evaluating the association between several chemokine/cytokine-related genes and immune response or survival. After this correction, a P value of less than 0.005 was considered statistically significant, ensuring a rigorous standard for our findings [42].

3. Results

3.1. Demographic and clinical data

The demographic and clinical details of the 188 UM patients whose data was retrieved are outlined in Table 1. The median duration of follow-up, extending from the date of diagnosis to either the censored date or the occurrence of death, was 2.2 years, with an interquartile range of 1.3–3.3 years.

3.2. Mutations associated with uveal patient survival

In our analysis of 188 tumor samples from uveal melanoma (UM) patients, we identified the five most frequently mutated genes—GNAQ (46.8 %), GNA11 (46.3 %), BAP1 (26.6 %), SF3B1 (20.7 %), and EIF1AX (12.8 %)—and evaluated their potential association with survival outcomes, as detailed in Table 2. As compared to patients with wild-types, those with mutated GNAQ or SF3B1 gene had better PFS (GNAQ:HR = 0.44, 95 % CI = 0.20–0.96, P = 0.039; SF3B1: HR = 0.46, 95 % CI = 0.17–1.20, P = 0.112, non-significant), DSS (GNAQ: HR = 0.40, 95 % CI = 0.16–1.00, P = 0.049; SF3B1: HR = 0.11, 95 % CI = 0.02–0.85, P = 0.034), and OS (GNAQ: HR = 0.38, 95 % CI = 0.18–0.78, P = 0.008; SF3B1: HR = 0.06, 95 % CI = 0.01–0.46, P = 0.007) in univariate analysis. However, compared with wild-type BAP1, those with mutated tumor suppressor gene BAP1 had worse PFS (HR = 3.21, 95 % CI = 1.49–6.89, P = 0.003), DSS (HR = 2.90, 95 % CI = 1.22–6.88, P = 0.016), and OS (HR = 2.83, 95 % CI = 1.43–5.58, P = 0.003) in univariate analysis (Table 2, Fig. S1). No genetic mutation was significantly associated with DFS. For DFS, PFS and DSS, we didn't have sufficient samples with clinical phenotype data available (DFS: 30 samples with 5 events; PFS: 36 samples with 14 events; DSS: 37 samples with 7 events), and hence we only ran multivariable analysis for OS (total 74 observations with 17 events available). Only BAP1 gene mutation remained significant for OS (HR = 3.65, 95 % CI = 1.14–11.73, P = 0.030, Table 2) after adjusting for age, sex, and disease stage. Our results suggested that mutated genes may contribute significantly to disease prognosis and survival outcome in uveal patients.

3.3. No tumor mutations associated with immune cell infiltrates in UM tumors

To delve into the mechanisms by which mutated genes influence survival outcomes, we conducted an analysis of the top five mutated genes associated with uveal melanoma (UM) using 80 tumor samples from TCGA. We compared the immune cell infiltrates between samples with mutated genes and those with wild-type counterparts. Our findings revealed no significant difference in immune scores between samples with mutated genes and those with wild-type tumors (all P-values >0.05, as shown in Table S1).

These results imply that the impact of mutated tumor genes on disease progression may be exerted through pathways other than modulation of the antitumor immune response, or it could be that the sample size in the current study was insufficient to achieve statistical significance. This insight opens up further avenues of investigation to identify alternative mechanisms by which these mutated genes contribute to the progression and outcome of UM [24,43].

Table 1
Demographic and clinical factors in 188 UM patients.

Characteristics	Dataset (N = 188)
Age at diagnosis, years	
Median	64.0
Interquartile range	53.0–73.0
Female, n (%)	50/107 (46.7)
GNAQ mutation, n(%)	88/188 (46.8)
GNA11 mutation, n(%)	87/188 (46.3)
BAP1 mutation, n(%)	50/188 (26.6)
SF3B1 mutation, n(%)	39/188 (20.7)
EIF1AX mutation, n(%)	24/188 (12.8)
Stage III/IV at diagnosis, n (%)	44/80 (55.0)
Follow-up time from diagnosis to disease Relapse or Censoring, years	
Median	2.0
Interquartile range	(1.0–3.1)
Follow-up time from diagnosis to death or Censoring, Years	
Median	2.2
Interquartile range	(1.3–3.3)
Recurrence among all patients, n (%)	13/60(21.7)
Death, n (%)	46/160 (28.8)
Death from UM, n (%)	21/80 (26.3)

Table 2
Top 5 genetic mutations and uveal patient outcome.

Variable	DFS[1]			PFS[1]				DSS[a]			OS[a]			OS[b]	
	HR	95 % CI	*P*	HR	95 % CI	*P*	HR	95 % CI	*P*	HR	95 % CI	*P*	HR	95 % CI	*P*
GNAQ(ref = WT)	0.38	0.12–1.18	0.094	0.44	0.20–0.96	**0.039**	0.40	0.16–1.00	**0.049**	0.38	0.18–0.78	**0.008**	0.47	0.16–1.40	0.175
GNA11(ref = WT)	2.81	0.91–8.65	0.072	1.90	0.88–4.07	0.101	1.71	0.72–4.05	0.222	1.77	0.90–3.49	0.098	1.38	0.50–3.86	0.536
BAP1(ref = WT)	2.31	0.70–7.61	0.170	3.21	1.49–6.89	**0.003**	2.90	1.22–6.88	**0.016**	2.83	1.43–5.58	**0.003**	3.65	1.14–11.73	**0.030**
SF3B1(ref = WT)	0.67	0.21–2.21	0.515	0.46	0.17–1.20	0.112	0.11	0.02–0.85	**0.034**	0.06	0.01–0.46	**0.007**	0.40	0.09–1.69	0.210
EIF1AX(ref = WT)	–	–	–	0.22	0.03–1.62	0.137	–	–	–	0.27	0.04–1.98	0.198	–	–	–

DFS: disease-free survival; PFS: progression-free survival; DSS: disease specific survival; OS: overall survival. Boldface type indicates statistical significance.

[a] Univariate analysis.

[b] Multivariable analysis with adjustment for age at diagnosis, sex and disease stage. Only performed for OS due to small sample size.

3.4. Chemokines/cytokines associated with lymphocyte infiltrates in UM tumors

We employed the TIMER2.0, EPIC, and CIBERSORT-ABS deconvolution algorithms to evaluate immune cell populations and tumor purity using RNA-seq data from the UM TCGA cohort [14–16]. This analysis allowed us to examine the relationship between cytokine/chemokine gene expression and both tumor purity and T cell proportions (Table 3). Our focus was particularly on cytotoxic T cell populations, given their capacity to directly target and eliminate cancer cells, and their known correlation with favorable clinical outcomes in various tumor types [9–12].

Our findings indicated that the gene expression levels of PDCD1 and CTLA4 were predictive of the $CD8^+$ T cell subset in UM, with significant correlations (PDCD1: $r^{\wedge}2 = 0.30$, $P = 8.05 \times 10^{\sim}{-3}$; CTLA4: $r^{\wedge}2 = 0.28$, $P = 3.41 \times 10^{\sim}{-2}$, as per Table 3). Additionally, the cytokines and chemokines presented in Tables 3 and 4 (excluding CX3CL1) showed a positive relationship with PDCD1, CTLA4, $CD8^+$ T cells, and Tregs within the tumor specimens. Conversely, they were inversely related to B cells and dendritic cells ($P < 0.05$ after Bonferroni adjustment). Notably, no significant associations were found between these chemokines/cytokines and tumor purity or with $CD4^+$ T cells, macrophages, neutrophils, or NK cells in the UM patient samples ($P > 0.05$ after Bonferroni adjustment, as detailed in Tables 3 and 4). The data suggest a significant role for these cytokines and chemokines in modulating the tumor immune response and inflammation, potentially influencing the therapeutic landscape and patient outcomes in UM.

3.5. Association of elevated cytokines and chemokines with Impaired survival in uveal melanoma

Our analysis revealed that elevated levels of PDCD1, TNF, IL6, CXCL9, and CXCL10, which are cytokines and chemokines associated with immune response, were predictive of reduced overall survival (OS) in uveal melanoma (UM) patients within the TCGA dataset ($P < 0.05$ after Bonferroni adjustment; Table 3). These significant results indicate that an augmented presence of cytokines/chemokines related to $CD8^+$ T cells may be associated with adverse survival outcomes in UM.

3.6. $CD8^+$ T cell infiltration and its correlation with poor prognosis in uveal melanoma

In our analysis of TCGA patients, we observed that increased $CD8^+$ T cell populations correlated with reduced overall survival (OS) in univariate analysis, with a hazard ratio (HR) of 11.41 and a 95 % confidence interval (CI) of 1.66–78.46, which was statistically significant ($P = 0.013$; as detailed in Table 5 and Fig. 1). This association persisted and remained significant even after adjusting for age, sex, disease stage, and tumor purity, with an HR of 12.77 and a 95 % CI of 1.28–127.37 ($P = 0.030$; Table 5). No significant correlations were found between other immune cell subsets and OS (data not shown).

These findings indicate that heightened infiltration of $CD8^+$ T cells, which are known as cytotoxic immune cells, and the associated cytokines/chemokines within the tumor microenvironment, may be indicative of a more aggressive disease course and poorer patient outcomes in uveal melanoma. The role of $CD8^+$ T cells in UM appears to diverge from their role in other cancer types [9–12], although the underlying mechanisms contributing to this difference are not yet fully understood and warrant further investigation.

4. Discussion

In our study, we conducted a comprehensive analysis of the impact of genetic mutations on uveal melanoma (UM) progression using data from 188 patients in cBioPortal. Subsequently, we utilized deconvolution tools to estimate immune cell subsets in a cohort of 80 UM patients from The Cancer Genome Atlas (TCGA) based on RNA-seq data. Our initial findings indicated that patients with mutations in GNAQ or SF3B1 had improved outcomes, whereas those with mutations in the BAP1 tumor suppressor gene experienced

Table 3
Relationship between cytokine or chemokine gene expression levels and tumor immune response or overall survival in 80 patients with UM whose sequencing data were available in The Cancer Genome Atlas.

Gene	PDCD1[a]		CTLA-4[a]		Tumor purity[a]		$CD8^+$ T cell subset[b]		Overall survival[c]		
	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*	HR[d]	95 % CI[d]	*P*
PDCD1	–	–	0.66	**4.98E-11**	0.23	4.20E-02	0.30	**8.05E-03**	1.74	1.25–2.41	**1.00E-03**
CTLA4	0.66	**4.98E-11**	–	–	0.11	3.27E-01	0.28	3.41E-02	2.52	1.24–5.13	1.10E-02
IFNG	0.70	**1.69E-12**	0.71	**4.17E-13**	0.13	2.50E-01	0.34	**2.71E-03**	1.97	1.18–3.30	1.00E-02
TGFB1	0.55	**2.06E-07**	0.35	**1.68E-03**	0.06	5.87E-01	−0.01	9.51E-01	1.15	0.99–2.31	5.50E-02
TNF	0.69	**2.95E-12**	0.66	**9.60E-11**	0.19	9.92E-02	0.31	**6.47E-03**	3.15	1.44–6.86	**4.00E-03**
IL6	0.51	**2.72E-06**	0.54	**3.49E-07**	0.09	4.29E-01	0.45	**4.01E-05**	10.51	3.05–36.13	**<1.00E-03**
IL10	0.69	**3.35E-12**	0.57	**5.01E-08**	0.01	9.48E-01	0.19	9.41E-02	3.27	0.86–12.44	8.20E-02
CX3CL1	0.10	3.85E-01	−0.02	8.61E-01	0.73	1.12E-01	0.02	8.67E-01	1.16	0.75–1.81	5.03E-01
CXCL9	0.81	**4.31E-19**	0.69	**4.25E-12**	0.20	8.03E-02	0.32	**4.42E-03**	1.39	1.16–1.68	**1.00E-03**
CXCL10	0.81	**6.51E-19**	0.67	**3.79E-11**	0.21	6.47E-02	0.36	**1.23E-03**	1.39	1.13–1.71	**2.00E-03**

[a] Spearman correlation test.
[b] Purity-corrected partial Spearman correlation.
[c] Univariate survival analysis.
[d] HR: hazards ratio; CI: confidence interval.Boldface type indicates statistical significance.

Table 4
Relationship between cytokine or chemokine gene expression levels and other immune cells estimated with TIMER in 80 patients with UM whose sequencing data were available in The Cancer Genome Atlas.

Gene	B cell subset[a]		$CD4^+$ T cell [a]		Dendritic cell[1]		Macrophage[1]		Neutrophil[a]		NK cell [b]		Tregs[1,c]	
	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*
PDCD1	−0.47	1.94E-05	0.03	8.00E-01	−0.33	3.12E-03	0.15	1.92E-01	−0.04	7.36E-01	0.16	2.55E-01	0.57	6.58E-08
CTLA4	−0.28	1.40E-02	0	9.84E-01	−0.19	1.02E-01	0.14	2.23E-01	−0.03	7.75E-01	0.04	7.22E-01	0.45	**4.53E-05**
IFNG	−0.29	9.57E-03	−0.07	5.54E-01	−0.20	8.02E-02	−0.01	9.04E-01	−0.10	3.97E-01	0.08	4.78E-01	0.40	**2.65E-04**
TGFB1	−0.24	3.92E-02	0.03	8.23E-01	0.10	4.08E-01	0.08	5.16E-01	0.04	7.36E-01	0.26	2.20E-02	0.42	**1.42E-04**
TNF	−0.33	**3.62E-03**	−0.07	5.28E-01	−0.21	6.13E-02	0.08	4.99E-01	−0.06	5.89E-01	0.20	7.80E-02	0.45	**3.63E-05**
IL6	−0.17	1.31E-01	−0.15	1.96E-01	−0.41	**1.94E-04**	0.07	5.66E-01	−0.09	4.55E-01	0.01	9.61E-01	0.14	2.38E-01
IL10	−0.31	**6.74E-03**	0.11	3.54E-01	−0.19	9.08E-02	0.14	2.38E-01	0.18	1.22E-01	0.08	4.74E-01	0.44	**5.96E-05**
CX3CL1	0.12	3.07E-01	0.23	4.75E-02	−0.11	3.57E-01	−0.21	6.30E-02	−0.07	5.54E-01	−0.02	8.99E-01	0.27	1.57E-02
CXCL9	−0.40	**2.89E-04**	0.01	9.06E-01	−0.30	7.99E-03	0.13	2.70E-01	−0.04	7.23E-01	0.14	2.24E-01	0.44	**6.14E-05**
CXCL10	−0.33	**3.45E-03**	0.04	7.63E-01	−0.36	**1.33e-03**	0.09	4.35E-01	−0.03	7.98E-01	0.17	1.33E-01	0.48	**1.22E-05**

[a] Purity-corrected partial Spearman correlation.
[b] Estimated with EPIC, without adjustment for tumor purity.
[c] Estimated with cibersort-absBoldface type indicates statistical significance.

Table 5
CD8+T cell and other clinical variables associated with overall survival in 80 patients with UM whose sequencing data were available in The Cancer Genome Atlas.[a].

Variable	Univariate: $CD8^+$ T cell subset			Multivariable: $CD8^+$ T cell subset		
	HR	95 % CI	*P*	HR[a]	95 % CI[a]	*P*
$CD8^+$ T cell subset	11.41	1.66–78.46	**0.013**	12.77	1.28–127.37	**0.030**
Purity	–	–	–	7.54	0.59–97.31	0.121
Age	–	–	–	1.04	1.00–1.08	**0.043**
Male sex	–	–	–	1.34	0.53–3.39	0.534
Stage 3	–	–	–	1.28	0.49–3.34	0.612
Stage 4	–	–	–	40.10	3.64–441.53	**0.003**

[a] HR: hazards ratio; CI: confidence interval. Boldface type indicates statistical significance.

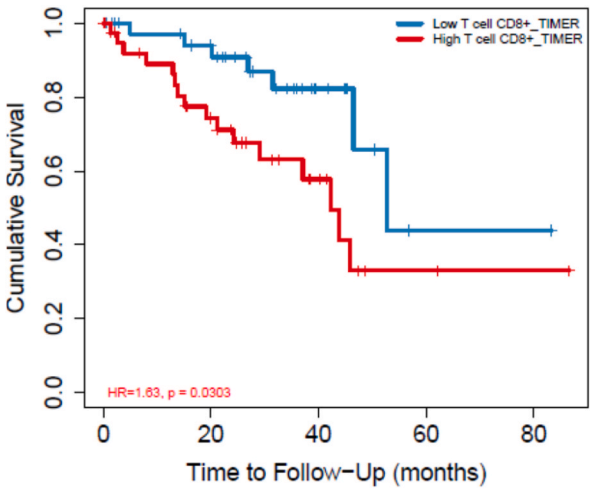

Fig. 1. $CD8^+$ immune score and overall survival. Cut-off was performed at median level.

worse outcomes compared to those with wild-type genes. We identified a correlation between various cytokines and chemokines and the immune response, particularly with the $CD8^+$ T cell population, which is a key player in the antitumor immune response. Furthermore, we observed that increased levels of $CD8^+$ immune cells and associated chemokines/cytokines were associated with reduced overall survival (OS) in UM patients. These results underscore the importance of systematically examining genetic mutations and chemokines/cytokines related to lymphocyte infiltration. Such investigations may aid in identifying UM patients who are likely to have different disease outcomes and prognostic trajectories. This approach holds promise for the development of more targeted and effective therapeutic strategies for UM.

The BAP1 gene, located on chromosome 3p21.1, is well-recognized for encoding a nuclear ubiquitinase that plays a crucial role in the epigenetic regulation of chromatin and maintaining genomic stability [44]. While our study did not uncover new insights into the BAP1 gene itself, our analysis confirms previous findings that mutations in BAP1 occur early in the development of uveal melanoma (UM) and are associated with the dissemination of micrometastases [45], indicating their potential role in early metastatic processes. This reaffirms the established notion that loss-of-function mutations in BAP1 are linked to the metastatic phenotype in UM and are indicative of a poorer disease-free and disease-specific survival [46,47]. Our findings, consistent with the current literature, further substantiate the negative prognostic impact of BAP1 mutations in UM. As such, the BAP1 gene remains a significant biomarker for patient outcomes, and its mutational status continues to be valuable for guiding clinical management and therapeutic decision-making in individuals with UM.

Our study found that UM patients with GNAQ or SF3B1 mutations had improved PFS, DSS, and OS. This is consistent with the findings of Terai et al., who also reported that SF3B1 mutations are associated with better OS, and that GNAQ Q209P mutants have a longer OS compared to GNAQ Q209L mutants [46]. These observations suggest that somatic mutations within the tumor genome can lead to the expression of neoantigens, which are recognized by the immune system, potentially triggering an enhanced antitumor immune response and contributing to improved patient outcomes. The presence of these neoantigens opens up the possibility of targeting them to boost antitumor immunity, offering a pathway for personalized cancer immunotherapy. This approach may be particularly beneficial for patients who derive limited benefits from traditional chemotherapy or radiotherapy. To delve deeper into the mechanisms by which these mutated genes influence survival outcomes, we analyzed the top five mutated genes in UM within 80 TCGA tumor samples and compared the immune cell infiltrates between mutated and wild-type samples. However, no significant differences in immune scores were observed between the mutated and wild-type samples. This lack of significance may be attributed to

the small sample size [48]. Given these findings, further research is merited to more definitively explore the relationship between gene mutations and patient outcomes in a larger cohort of UM patients. Additionally, functional experiments to assess the specific roles of mutated GNAQ or SF3B1 in disease progression are necessary to complement and expand upon the current study's findings, although such experiments fall outside the scope of our present investigation.

Identifying cytokines and chemokines that are associated with immune responses can provide insights into the mechanisms that regulate these responses within the tumor microenvironment (TME). This information can offer more precise details about T cell activation or inhibition than what is typically provided by traditional pathologist-assessed tumor-infiltrating lymphocyte (TIL) evaluations. Utilizing the TIMER deconvolution analysis to estimate immune cell populations, Li et al. highlighted key chemokine-receptor networks involved in immune infiltration across various tumor types. Their research found that high levels of $CD8^+$ T cells were linked to a variety of chemokine-receptor pairs, such as CCL3,4,5–CCR1,5 and XCL1,2–XCR1, while macrophage infiltration was associated with the CXCL12–CXCR4 pair in several cancers, including head and neck, thyroid, stomach, and colon [12]. Our examination of chemokines and cytokines related to inflammation and tumor immune responses revealed multiple associations with $CD8^+$ T cell subsets. These findings suggest that these biomarkers could be targeted to enhance $CD8^+$ T cell responses or to identify patients who may benefit from immunotherapeutic approaches. Consistent with this, numerous studies have indicated that the presence of $CD8^+$ T cells and their associated chemokines/cytokines in the TME is a positive predictor of clinical outcomes in various tumor types [9–12]. However, in contrast to their roles in other tumor contexts, molecular profiling of uveal melanoma with tumor-infiltrating lymphocytes in our study and previous research [32] suggests that $CD8^+$ regulatory T cells and certain chemokines contribute to an immunosuppressive TME and may advance disease progression in uveal melanoma.

Interestingly, our analysis did not identify any direct association between specific tumor mutations and immune cell infiltrates in UM tumors, indicating that the mechanisms governing immune response in UM may be distinct from other cancer types. Our results, when juxtaposed with those from other studies, particularly those by Sun et al. and Hurdogan et al., bring to light the dualistic role of $CD8^+$ T cells in UM [49,50]. Contrary to their generally accepted role as mediators of effective antitumor immunity, high levels of intratumoral $CD8^+$ T cells were correlated with a poor prognosis in UM, a finding that challenges the conventional understanding of these immune cells' function. Delving deeper into the adaptive immune resistance mechanisms, our study highlights the necessity of comprehending the tumor's evasion strategies against immune surveillance. The presence of specific genetic mutations, in conjunction with an immunosuppressive TME, may elucidate why an increased presence of $CD8^+$ T cells does not always translate into enhanced antitumor activity in UM. Different methodologies employed across these studies have yielded variable outcomes, emphasizing the complexity and heterogeneity inherent in UM's immune microenvironment.

We acknowledge the limitations inherent in our study, which, despite providing novel insights into the genetic and immunological underpinnings of uveal melanoma (UM), require further investigation. The single time-point RNA-seq analysis limits our ability to capture the dynamics of immune-cell populations and tumor heterogeneity across different metastatic sites. Additionally, our modest sample size may have constrained the detection of subtle genetic-immune associations, suggesting the need for larger cohorts to discern such relationships fully. The cross-sectional design also precludes assessment of temporal changes in immune cell composition and cytokine expression, pivotal for understanding disease progression and therapeutic responses. Our findings, though consistent with some literature, challenge the conventional role of immune cells in tumor progression and highlight the necessity for longitudinal studies with diverse methodologies to confirm and expand upon our results. Furthermore, the reported associations between cytokines in the tumor microenvironment and UM survival outcomes necessitate validation across multiple cohorts, emphasizing the preliminary nature of our conclusions and the need for additional well-designed studies to provide broader substantiation. Overall, our study indicates that distinct expression patterns of tumor cytokines significantly influence the immune response in UM. Additionally, our results highlight that genetic mutations, chemokines/cytokines, and the immune response each have independent effects on the survival outcomes of UM patients. These insights contribute to a more comprehensive understanding of the complex interplay between the tumor microenvironment and UM progression, which is crucial for developing targeted therapeutic strategies.

Ethics statements

Patient consent for publication, not applicable.

Ethics approval

Not applicable.

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Data availability statement

Datasets related to this article can be extracted at [https://www.cbioportal.org/] and [https://portal.gdc.cancer.gov/projects/TCGA-UVM], hosted at [National Cancer Institute GDC Data Portal]

CRediT authorship contribution statement

Yong liu: Writing – review & editing, Conceptualization. **Yeen Huang:** Formal analysis. **Chengzhi Zhao:** Project administration, Formal analysis. **Xinke Zhou:** Writing – review & editing, Writing – original draft, Conceptualization. **Jiachun Lu:** Writing – original draft, Visualization. **Shenying Fang:** Supervision, Project administration, Funding acquisition.

Declaration of competing interest

☑ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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