

Omics analysis of uveal melanoma: Leukocyte gene signatures reveal novel survival distinctions and indicate a prognostic role for cytolytic activity scoring

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Abstract

Objective: The significant metastatic potential of uveal melanoma (UVM) lends to high mortality. Even with successful local tumor treatment, many patients will develop metastatic disease. The present study aims to elucidate the relationship between tumor-infiltrating immune cell (TIIC) diversity and survival to identify potential therapeutic targets and improve UVM prognosis.

Methods: Bulk deconvolution was used to determine the relative proportions of 22 hematopoietic TIIC from 80 UVM tumor samples. Cytolytic activity (CYT) was determined, and associated survival probabilities were mined using time-to-event data. Nominal *P*-values were subjected to FDR correction.

Results: High relative abundance of tumor-infiltrating naïve B cells, resting memory CD4⁺ T cells, and monocytes correlated with better overall and disease-free survival probability. Low relative abundance of CD8⁺ T cells correlated with better overall survival and disease-free survival probability. CYT correlated positively with relative abundance of naïve B cells, resting memory CD4⁺ T cells, and monocytes. CYT correlated negatively with relative abundance of CD8⁺ T cells.

Conclusion: Infiltrating naïve B cells, resting memory CD4⁺ T cells, monocytes, and CD8⁺ T cells are potential therapeutic targets in UVM that warrant further investigation. High CYT estimates associate with worse UVM survival outcomes.

Keywords: CD4⁺, UVM, Cytolytic activity, B cells, Infiltration

Introduction

Melanomas are a vast family of cancers derived from malignant melanocytes with significant locational diversity, including the skin, mucous membranes, and ocular region [1]. In a study of 84,836 cases of melanoma, Chang et al. found that up to 5% of such neoplasms are derived from the eye [1]. Among these cases, uveal melanoma (UVM) is the most common, accounting for up to 83% of all ocular melanomas [1,2]. Furthermore, as the most common primary ophthalmic malignancy in adults and with a median presentation age of 59 to 62 years of age, UVMs have a disproportionately high socio-economic impact [3]. This effect is compounded by the pathology's high propensity for metastasis [4]. Currently, up to 50% of patients with UVM develop metastatic disease, most frequently affecting the liver [5]. In a retrospective medical record review of 8,033 eyes, Shields et al. identified increased age and tumor size as strong positive correlates with the probability of metastasis [6].

Modern treatment options for primary UVM aimed at mitigating metastatic disease include enucleation and radiation therapy. However, these approaches have been found to have limited and carryable success. In a 20-year retrospective chart review, Kujala et al. determined that 30-50% of patients who underwent enucleation and radiation therapy developed metastases, even after successful local treatment of the primary tumor [5]. This suggests the presence of dormant micro-metastases that form during early tumorigenesis [5,7]. Consequently, understanding the

immunological underpinnings can help devise therapies for UVM. In this vein, tumor-infiltrating immune cells (TIICs) have demonstrated prognostic benefit across many cancer types [8]. Existing studies investigating TIIC show that an increased abundance of infiltrating T cells is associated with worse clinical outcomes in UVM [9-11]. However, these experiments utilized immunohistochemistry as their method of detection, limiting the accuracy of their analysis. In order to detect TIIC in small quantities and elucidate complex and related cell types, a different approach is necessary [12].

CIBERSORTx (<https://cibersortx.stanford.edu/>) is a well-established deconvolution tool to accurately estimate cell-type abundances without single-cell transcriptome data. This algorithm can be applied to investigate TIIC diversity via *in silico* quantification, supporting downstream clinical correlate analysis [13]. This method has improved clinical efficacy and consistently replicates conventional flow cytometry results, enabling researchers to rapidly elucidate tissue cell expression [12,14-15]. Improving UVM treatment options requires a strong understanding of the immunogenic landscape, particularly for treatment-resistant populations. Here we utilize CIBERSORTx to quantify 22 leukocytes and explore their individual contributions to overall and disease-free UVM survival. This evaluation contributes to a growing body of literature demonstrating application of an effective alternative analysis to immunohistochemistry while also identifying potential immunological therapeutic targets to improve UVM patient outcomes.

Methods

Data acquisition

Level 3 bulk mRNASeq data and corresponding survival data representing eighty (80) UVM patients were obtained from the Broad Institute GDAC Firehose portal (<https://gdac.broadinstitute.org/>). The gene-level transcripts per kilobase million (TPM) values, defined as $1e6 \times$ scaled RSEM estimates, were used for downstream matrix deconvolution.

Computing tumor-infiltrating immune cell fractions

CIBERSORTx, a well-established RNA deconvolution algorithm, was used to quantify immune cell proportions. A curated

leukocyte gene signature matrix (LM22), comprised of 547 immune cell type-specific genes, was used to estimate the relative abundance of 22 mature human hematopoietic populations. CIBERSORTx was run at 1000 permutations with bulk-mode batch correction.

Statistical analysis

Deconvolution results were considered statistically significant at CIBERSORTx p-value <0.05, derived from Monte Carlo sampling across all LM22 gene sets. Kaplan-Meier (KM) analysis utilized median immune cell relative fraction to assess for distinct survival probabilities. Overall survival (OS) was defined as the length of time from date of diagnosis to date of any-cause death. Disease-free survival (DFS) was defined as the length of time post-treatment that a patient survives without cancer recurrence. Cell types with median relative fraction of 0% were excluded from downstream analysis. Q-values <0.01 were deemed statistically significant. Cytolytic activity (CYT), a relative measure of cancer immunity, was defined as the log-average of *GZMA* and *PRF1* mRNA expression levels [16]. Kendall rank correlation was evaluated with hierarchical clustering using R package 'corrplot' [17]. All analyses were conducted using R version 4.1.2.

Results

UVM TIIC landscape

Batch-corrected deconvolution was achieved using an established omics approach (Figure 1). M2 macrophages represented, on average, 41% of the tested immune cell population; B cells accounted for <5% of the total TIIC population; eosinophils and neutrophils combined to represent 0.5% of the tumor immune cell environment. See Table 1 for a tabulation of the 22-TIIC deconvolution output.

TIIC associations with survival

The association of isolated tumor-infiltrating leukocyte populations with overall survival was assessed by KM estimation to identify the greatest contributors to UVM patient outcomes (Figure 2, top row). Levels of naïve B cells, resting memory CD4⁺ T cells, and monocytes in the tumor microenvironment correlated positively with better probabilities of overall survival. Higher proportion of CD8⁺ T cells were negatively related with UVM overall survival outcomes. Next, we analyzed disease-free progression among the

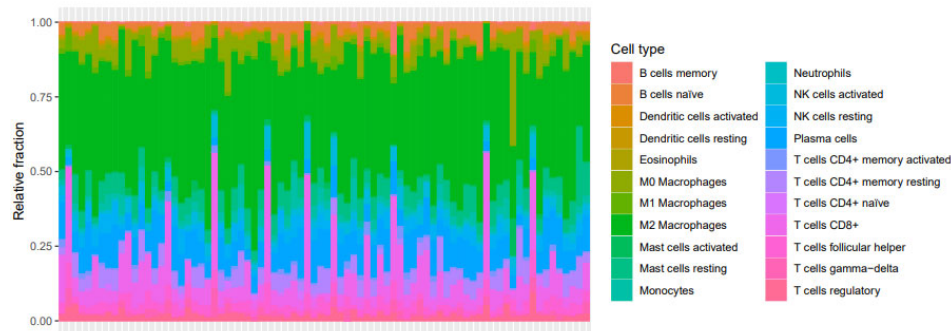


Figure 1.

Table 1. Relative fraction of 22 TIICs, based on Figure 1.		
Tumor-infiltrating Immune Cell Type	Relative abundance (%)	CIBERSORT p-value
Naïve B cells	3.570133	0
Memory B cells	0.297648	0
Plasma cells	9.528872	0
CD8 ⁺ T cells	10.91962	0
Naïve CD4 ⁺ T cells	0.297768	0.004
Resting memory CD4 ⁺ T cells	4.235605	0
Activated memory CD4 ⁺ T cells	0.520088	0.006
Follicular helper T cells	3.475006	0.005
Regulatory T cells	2.291469	0.006
Gamma-delta T cells	0.97832	0
Resting NK cells	3.082866	0
Activated NK cells	2.682618	0
Monocytes	2.844411	0
M0 Macrophages	5.468126	0
M1 Macrophages	1.525249	0.001
M2 Macrophages	41.04857	0
Resting dendritic cells	0.710383	0
Activated dendritic cells	0.196516	0.013
Resting mast cells	5.461177	0
Activated mast cells	0.348891	0
Eosinophils	0.401973	0
Neutrophils	0.114694	0.003

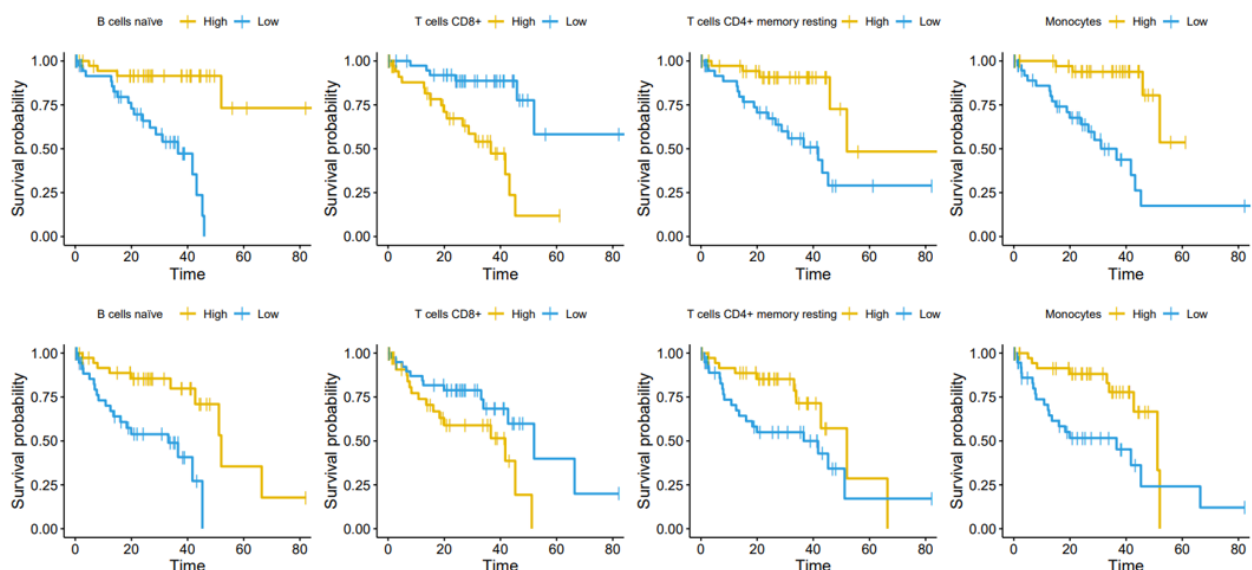


Figure 2.

THIC subpopulation with significant overall survival interaction to explore whether similar trends are obtained (Figure 2, bottom row). All THICs with previously identified contributions to overall survival retained their directionality and remained statistically significant.

THIC correlation and spatial representation of cytolytic activity

Because CYT is understood to associate variably with survival outcomes across different cancer types, we sought to investigate its

interplay with distinct UVM THIC subpopulations, which, to the authors knowledge represents a first-use case (Figure 3A). Among the set of THICs which revealed positive correlation with UVM survival probability, CYT correlated negatively. Further, CYT correlated positively with relative fraction of CD8+ T cells, which reported negative association with UVM survival probability. Principal component (PC) analysis of the first two PC axes representing the (Figure 3B). See Table 2 for the Kendall rank correlation coefficient results.

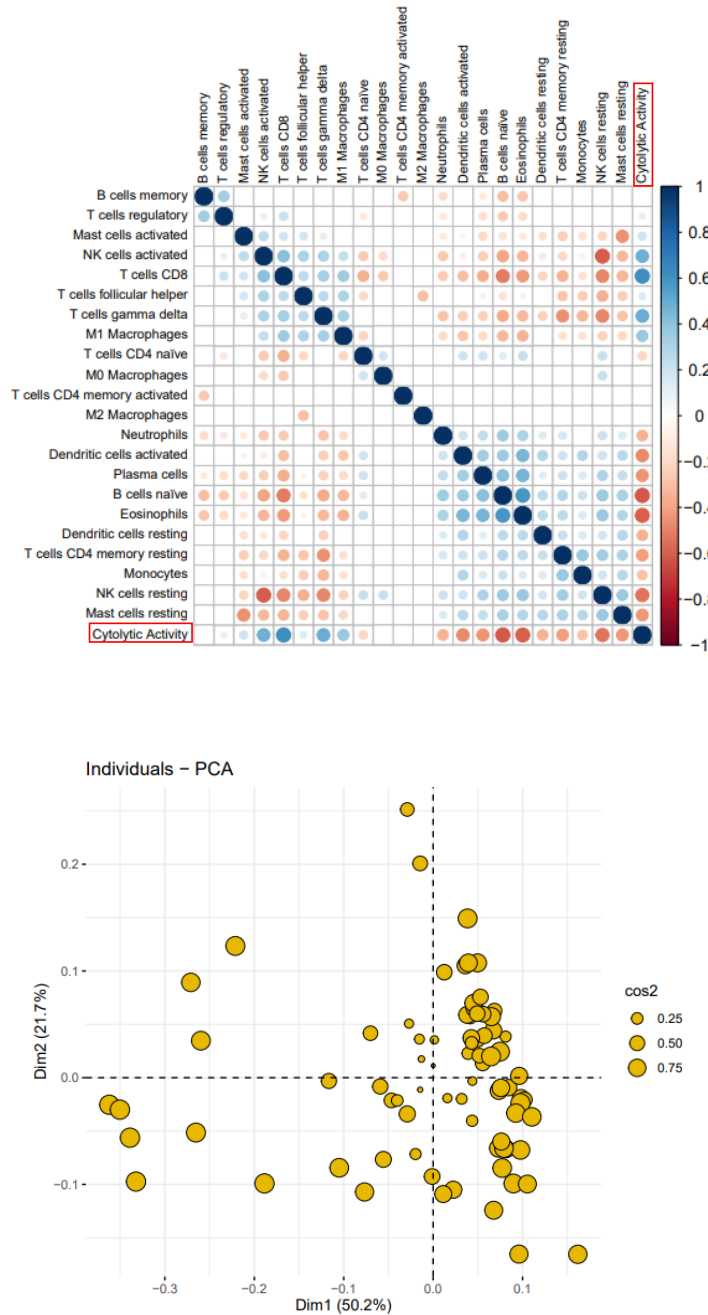


Figure 3.

Table 2. Kendall's Tau results correlating CYT and levels of prognostic TIICs.		
Tumor-infiltrating Immune Cell Type	tau-B value	Survival association
CD8 ⁺ T cells	0.620 (Strong)	Negative
Naïve B cells	-0.614 (Strong)	Positive
Resting memory CD4 ⁺ T cells	-0.415 (Strong)	Positive
Monocytes	-0.288 (Moderate)	Positive

Discussion

Transcriptome analysis revealed a complex TIIC landscape with a predominance of M2 macrophages (Figure 1). M2 macrophages are produced by alternative activation of tumor-associated macrophages (TAMs) and promote tumor progression and metastasis [18]. Therefore, targeting TAM activation has proven as an effective tumor therapy [19]. Conversely, classically activated M1 macrophages exhibit anti-tumor behavior [20]. Among the examined UVM population, M1 macrophages observed a low relative TIIC abundance of <2%, whereas M2 macrophages represented 41% of the overall TIIC landscape (Figure 1, Table 1). In existing literature, plasticity between M1 and M2 macrophages has been found to be influenced by the tissue microenvironment. Consequently, microenvironmental modification has been identified as a potential therapeutic target [21].

Furthermore, score-based systems have been created to assess how the genomic landscape of neoplasms influences and is influenced by anti-tumor activity. The cytolytic activity score (CYT) has been documented as such a metric. CYT approximates for cancer immunity based on mRNA expression of two cytotoxic granules: granzyme A and perforin [16,22-23]. High CYT routinely implicates better survival due to activation of effector T and NK cells, but exception exists [24]. In the selected primary UVM population, cytolytic activity negatively correlates with the abundance of tumor-infiltrating naïve B cells (strong), resting memory CD4⁺ T cells (strong), and monocytes (moderate) (Figure 3B, Table 2). Cytolytic activity positively correlates with the abundance of CD8⁺ T cells, consistent with differentiation into cytotoxic T lymphocytes that produce cytotoxic granules.

Despite this association, our results demonstrate that a high density of CD8⁺ T cells decreases overall survival in UVM (Figure 2). This negative prognosis is consistent with previous literature that documents worse clinical outcomes with greater T-cell infiltration [9-11]. Staibano et al., suggest that CD8⁺ function may be impaired in UVM [25]. Building off this conclusion, McKenna and Previte hypothesized that CD8⁺ T cells with limited functionality accumulate and signal M2 macrophages that exhibit pro-tumor activity [26]. Looking beyond the primary tumor, T cells and macrophages represent the predominant infiltrate in liver metastases [27,28]. Our results also signal that overall survival is positively influenced by the increased presence of naïve B cells, resting memory CD4⁺ T cells, and monocytes (Figure 2). Monocyte heterogeneity yields some subtypes with pro-tumor properties, facilitating angiogenesis and metastasis, within both the classical and non-classical subsets [29]. Alternatively, anti-tumoral monocytes play roles in tumor cytotoxicity, metastatic prevention, recruitment of NK cells, and inhibition of Tregs [29]. Similar to TAMs, monocytes exhibit a high degree of plasticity that may serve as a target with the goal of enhancing antitumoral immunity [30].

The extent to which these findings can be generalized and interpreted is limited by certain experimental shortcomings. The cohort consisted of only 80 primary UVM samples with survival data available from the Broad Institute GDAC Firehose. A larger dataset would conceivably account for additional diversity in TIIC proportions. Additionally, the CIBERSORTx algorithm is unable to account for spatial tissue and cytoarchitecture. While CIBERSORTx can competently differentiate between monocytes and macrophages, the algorithm cannot distinguish different monocyte subsets. Subset classification is essential to understand the possible mechanisms of monocyte involvement in overall UVM survival. Still, the authors confer that the findings reported in the current study lend strong support for further omics investigation of CYT and isolated TIIC populations in the context of primary UVM.

Conclusion

Deconvolution algorithms have allowed the rapidly characterization and contextualization of the tumor microenvironment. Among the analyzed 22-member set of leukocytes, M2 macrophages were most abundant in the UVM tumor environment. KM results indicated that overall survival probabilities correlated with greater relative abundance of naïve B cells, monocytes, and resting memory CD4⁺ T cells. Infiltrating CD8⁺ T cells correlate with lower overall survival. Interestingly, cytolytic activity estimates positively correlated with levels of CD8⁺ T cells, and negatively correlated with levels of naïve B cells, resting memory CD4⁺ T cells, and monocytes. These data suggest that high CYT associates significantly with worse survival outcomes through abundance estimates of individual TIICs, supporting its prognostic value for UVM. The authors hope the findings shared here inspire future UVM research towards the benefit of identifying potential immunotherapy targets.

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