OncoPeptTUME™ — A novel in-silico approach to model the tumor microenvironment and predict treatment efficacy and long-term survival benefits for immunotherapy applications

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Abstract

Cancer immunotherapy is now established as a major therapeutic modality, and 70% of all cancer patients are estimated to receive some form of immunotherapy treatment as a part of their disease control by 2025. Cancer immunotherapy drugs elicit their anti-tumor immune response in a subset of the treated patients by activating CD8+ T cells and provide sustainable and long-lasting benefit in a few. Recently significant efforts have been devoted to understanding the factors that influence response to immuno-therapy or contribute to the development of resistance to therapy. While it is appreciated that many different tumor cell-intrinsic and extrinsic features, including the tumor microenvironment, driver gene mutations, host genetics, microbiome and environmental factors modulate response to immune checkpoint inhibitors , the tumor microenvironment ecosystem could be a major contributor in regulating response to immunotherapy and development of resistance. Ongoing efforts to characterize the tumor microenvironment to stratify patients for immunotherapy, and find biomarkers of response often use methods that are limited by 1) availability of adequate tumor tissue from needle biopsy material; 2) restricted set of cell surface and phenotypic markers to analyze the cellular composition with limited tissue availability, and 3) loss of tissue integrity during processing for downstream analysis. Recently, single-cell transcriptomics has enabled studies to analyze the heterogeneity in a population of cells from a tissue and define gene expression signatures in the tumor microenvironment , but the quality of data generated is still governed by the sample collection method and quality of RNA (determined by the presence of viable cells). Alternatively, genomic methods that use deconvolution to assess relative enrichment of different cells types can be utilized to understand the composition of the tumor microenvironment, but that approach can also be limited in utility by biases introduced by dependencies in the cell type . Taken together, a robust method of studying the tumor microenvironment to identify the molecular signature is still needed. To this end, MedGenome Inc has developed OncoPeptTUME™, a genomic solution that utilizes its highly cell-type specific proprietary minimal gene expression signature for 8 different immune cells. The expression of genes for a given signature was transformed to produce a cell-type specific immune score that was used to quantitate the relative proportion of cell types present in the complex tumor microenvironment. We present data to highlight a) how the proprietary gene expression signatures were generated and validated, b) robustness of our gene signatures compared to other existing methods in identifying cell types of interest c) utility of the OncoPeptTUME in defining immunogenicity (via immune score assignments) of tumors and predicting prognosis and long-term survival benefits based on the immune signatures of the tumors.

Workflow for tumor-microenvironment analysis using OncoPeptTUME

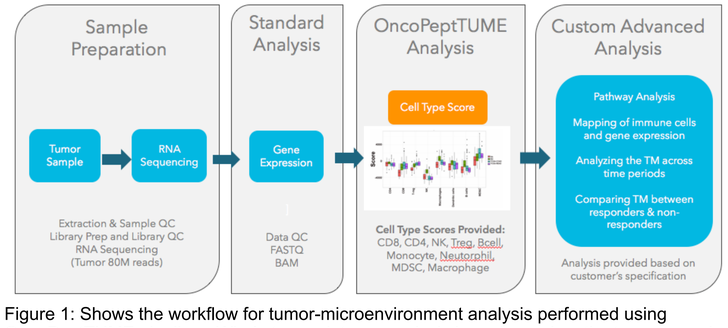

Figure 1: Shows the workflow for tumor-microenvironment analysis performed using OncoPeptTUME pipeline : Whole transcriptome analysis is performed on the tumor sample of interest and OncoPeptTUME analysis is performed to determine the immune score of the tumor based on the relative enrichment of 8 different types of immune cells in the tumor. Further analysis is performed to understand the pathways enriched in the tumors and

Building the Gene expression signatures for the OncoPeptTUME pipeline

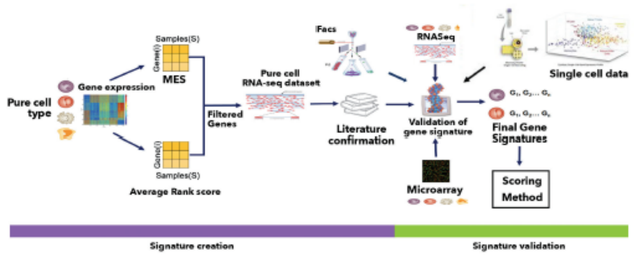

Figure 2: Workflow for building the minimal gene expression signatures (MGESPs) : Publicly available microarray gene expression data was used to calculate the Average rank score and MES scores (measures of specificity and plasticity of a gene in each of the pure cell types respectively). The signatures were further refined by curation and validated on an independent set of microarray and RNA-Seq data from pure cell populations.

Validation of gene expression signatures using publicly available gene expression datasets

Table 1 : shows the datasets that were used to generate the gene expression signatures

Immune Response		Innate						Adaptive					Total
Cell type		NK	Neutrophils	Monocytes	Macrophages	M2	M1	Treg	DC	CD8	CD4	B cell	
Building the signature		149	457	462		385		72	411	189	619	1545	4289
Validation	RNA seq	14	20	20	-	-	-	5	-	20	20	20	103
	Microarray	81	114	186	45	45	32	130	153	92	73	158	1445

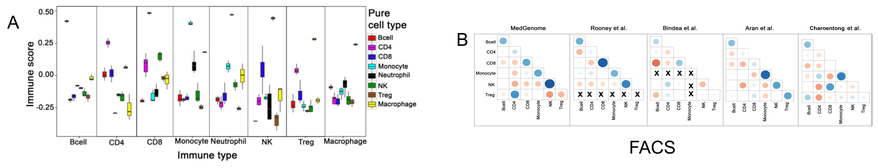

Figure 3: A) Validation of MGESPs on RNA-Seq data represented as boxplots with cells on the x-axis and immune scores on the y-axis. Each facet represents the immune score (Y-axis) calculated for a specific cell-type plotted on the X-axis. A higher score was observed for cognate cell type compared to non-cognate cells. B) Performance comparison of MGESPs with other published signatures on FACS data. Figure shows correlation between MGESP scores with FACS-based enumeration of cells. Size of the bubble indicates sample number, and blue circle represents positive correlation and red circle represents negative correlation.

OncoPeptTUME predicts immunogenicity of tumors in TCGA data

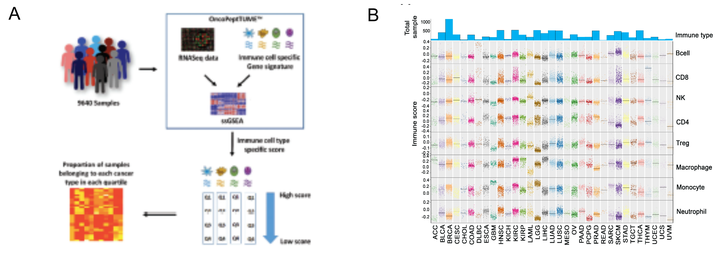

Figure 4: Comprehensive analysis of the immune landscape of 9640 tumors across 33 cancers using using OncoPeptTUME : A) Shows the OncoPeptTUME workflow to process 9640 TCGA datasets to identify cancers of the highest immune filtration for a particular immune cells type. MGESP derived score for each cell type was calculated for each of the tumors and arranged into quartiles. Q1 implies highest level of infiltration of a certain immune cell type into the tumor and Q4 implies lowest level of expression. B) Representation of the relative enrichment of 8 different immune cell types in all the different cancer type. Using this approach we identified some interesting immune co-infiltration signatures in different such as Kidney renal cell carcinoma (KIRC) known to be a immune-sensitive tumor had a high infiltration of all the immune cell types except Treg cells, whereas kidney renal papillary cell carcinoma (KIRP) showed lesser infiltration of most immune cell types. Interestingly, kidney chromophobe cancer (KICH) showed a high infiltration of NK cells and low infiltration of other cell types, previously reported by immunohistochemistry analysis. In conclusion, the analyses using OncoPeptTUME shed interesting insights into the immune landscape of different cancers.

OncoPeptTUME identifies effect of immune cell infiltration on long-term survival in TCGA cancer-types

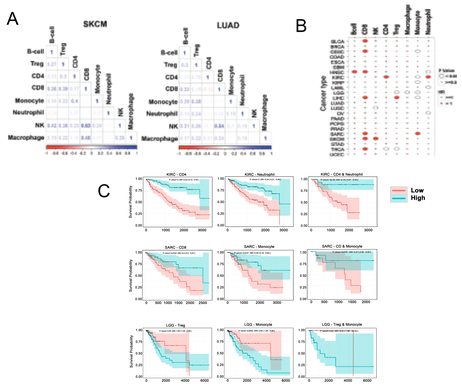

Figure 5: A) Representation of the correlation of immune infiltration seen in SKCM and LUAD cancers. B) Correlation between infiltration of different immune cells and patient survival. For each cancer survival benefit between the top and bottom 20% tumor samples infiltrated by specific immune cells was compared. Size of the bubble shows sample number, red and blue indicates good and poor prognosis respectively, and significant associations (p-value <.005) are shown. C) Effect of combined infiltration of two cell-types on patient survival represented as Kaplan Meir plots for selected cancers.

OncoPeptTUME can be utilized to stratify patients as responders and non- responders

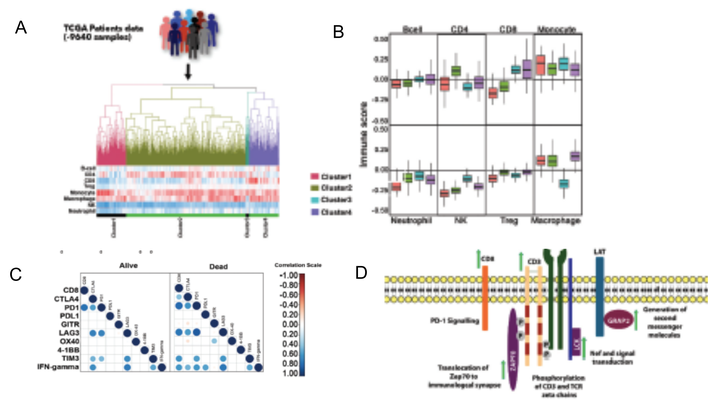

Figure 6: A) Clustering of TCGA patient samples using hierarchical clustering using immune scores derived using the minimal gene expression : Four major clusters are represented in different colors with their corresponding immune cell type infiltration represented as a heat-map below the dendro-gram. B) Boxplot showing the variation in the distribution of immune infiltration scores for each immune cell type across the four clusters. C) Correlation of expression between the infiltration of CD+ T-cells vs the anergic and exhaustion markers with the CD8+ T-cell in the two groups. E)Cartoon representation of the genes upregulated in the TCR signaling pathway in the alive subjects of cluster-4.

Conclusions

OncoPeptTUME is a superior tumor microenvironment analysis pipeline which utilizes highly specific minimal gene expression signatures of 8 different immune cells and can identify immune cell types with greater accuracy than other comparable methods.

OncoPeptTUME workflow overcomes the challenges associated with deconvulation and single-cell gene expression profiling methods to study the tumor microenvironment.

Tumor microenvironment analysis using this tool reveals interesting immune cell co-infiltration patterns and allows for clustering of the cancer datasets into quartiles for further identification of markers for stratification of patients for immunotherapy.

Tumor microenvironment analysis using this tool reveals a mechanism of poor prognosis of patients with tumors that have high CD8+ T cells by identifying enrichment of TCR signaling pathways in live versus dead patients , and enrichment of markers of exhausted T cells in dead patients.