Deconvolution of diverse immune cell populations within tumors using ACE Transcriptome

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Eric Levy[1], Sean M. Boyle[1], Pamela Milani[1], Shujun Luo[1], Michael Leipold[2], Holden T. Maecker[2], John West[1], Richard Chen[1]
[1]Personalis, Inc. | 1330 O'Brien Dr., Menlo Park, CA 94025
[2]Stanford University School of Medicine | 299 Campus Dr., Stanford, CA 94305

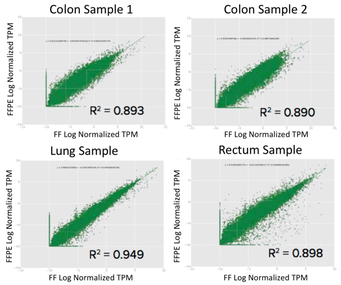
Contact:
eric.levy@personalis.com

Introduction

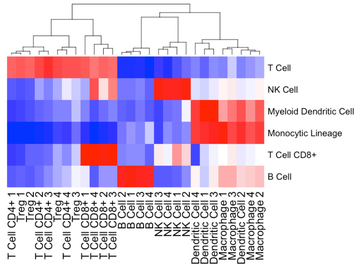
Comprehensive tumor immuno-genomic characterization is becoming an important tool for identifying new biomarkers correlated with patient response to immunotherapy. Both the abundance and composition of tumor-infiltrating immune cells have been associated with tumor progression and patient outcome. There is interest in using an accurate computational method leveraging NGS data to more comprehensively profile the abundances of immune cells in the tumor microenvironment. Through the use of marker genes that are expressed in a cell type specific manner, it is possible to computationally predict the abundances of multiple cell types in a mixed sample. We have used the ACE Cancer Transcriptome, from the ACE ImmunoID platform, to produce high-quality gene expression profiles of purified immune cells representing many lineages. These profiles were used to create reference signatures of immune cell type specific genes, enabling quantification of their cellular abundances.

ACE Cancer Transcriptome

The ACE Cancer Research Transcriptome has been specifically designed to produce high-quality transcriptome sequencing results from challenging FFPE samples. Through analysis of matched FF and FFPE tumor samples, we demonstrate that ACE is able to accurately quantify expression in FFPE samples. This is critical for ensuring that our deconvolution approach is accurate even when using degraded FFPE samples.



Sequencing of purified immune populations



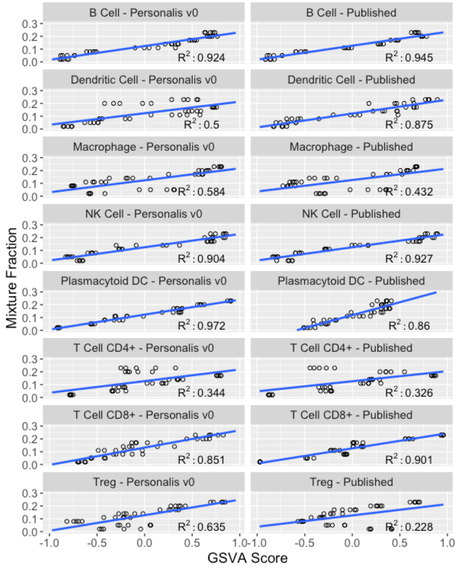
In order to generate reference profiles representing distinct immune populations, we obtained and sequenced purified immune cells from 4 separate donors each for 8 cell types. Gene set variation analysis[1] of published gene sets[2] for our samples gives us confidence in the quality of the purification and sequencing, enabling us to use these samples to generate high-quality reference profiles of these immune cell types.

Testing on *in silico* mixtures of immune cells

Creation of *in silico* mixtures and Personalis v0 gene sets

In order to develop the methodology to create reference expression profiles, we used an *in silico* mixture testing approach. For the test mixtures, we randomly selected 1 sample for each cell type, and combined a subset of reads from each sample at set fractions. These mixtures were then run through our transcriptome pipeline to quantify their expression. The remaining 3 samples for each cell type were then used to generate the reference of marker genes. Finally, we ran GSVA with our gene sets and gene sets curated from the literature[2-4] to test the performance of our platform and approach.

Tracking abundances of immune cells with a gene set approach

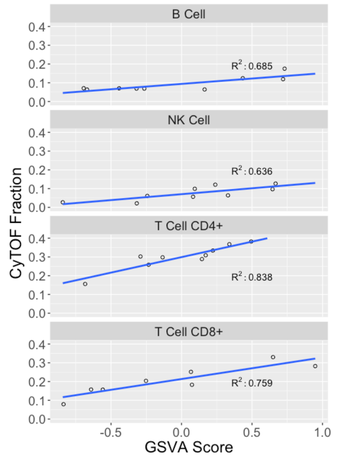


We see that by using our transcriptome with gene sets representing the immune cell types profiled, we are able to closely track the actual fractions of immune cells in the mixtures using GSVA scores.

In many cases, enrichment of gene sets based on our own reference samples are very closely aligned to the fractions in the mixtures. Through this testing, we are also able to identify cases where previously-published gene sets are informative on our platform.

Through further testing and validation of the approach, we will create a report providing profiling of the infiltration of a sample using ACE ImmunoID. However, expression results from our ACE Cancer Transcriptome currently allows for this analysis to be done by the customer.

Testing on PBMC samples



For initial testing of our approach on samples with diverse immune populations, we compared our expression profiling on 9 healthy PBMC samples with corresponding cellular abundances as measured by CyTOF.

We see that for cell types that have presence in the PBMCs, a gene set enrichment approach on the ACE Cancer Transcriptome is able to accurately track their abundances.

For future work, we are planning to test using additional orthogonal technologies. In addition, we will test more healthy PBMC samples, as well as real tumor samples, to verify that our approach is able to accurately predict abundances in actual tumor infiltrating immune cells.

Conclusion

While common lab approaches are used to profile tumor samples for the presence and enumeration of immune cell types, these approaches can be limited by the number of markers and throughput. Through NGS and an input of reference gene expression signatures, we can estimate the proportions of the cell types represented in the reference. The generation and validation of these reference signatures are critical for ensuring the accuracy of the results on a given platform. We have tested the accuracy of the deconvolution approach on mixtures of immune cells, as well as on PBMC samples with immune cells quantified CyTOF. Through these results, we show that deconvolution of TILs through our ACE Cancer Transcriptome can be a robust platform for elucidating the composition of immune cells in a tumor sample.

References:
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