Quantification of tumor-infiltrating immune cell populations with an augmented transcriptome

#4430

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Introduction

Comprehensive characterization of the tumor and tumor microenvironment (TME) can improve our understanding of tumor progression and treatment outcomes. In particular, quantification of the immune infiltrate has the potential to inform mechanisms of immune escape and predict response to therapy[1]. Standard experimental approaches exist to enumerate tumor-infiltrating immune cells, but they can have practical limitations of throughput, number of markers, or sample requirements. RNA sequencing can be used as a scalable solution to comprehensively profile the immune cell composition of the TME. By identifying marker genes that are expressed by specific immune cell types, we can create a computational method to quantify the abundance of these cell types in a mixed sample. However, care must be taken to verify that such computational analysis accurately reflects the underlying immune cell composition. Here, we utilize our transcriptome platform, ImmunoID NeXT, to develop and evaluate a methodology for immune cell quantification. We compare our transcriptome-based quantification to orthogonal measurements of immune cell abundance to ensure accuracy, and highlight the utility of the methodology by comparing results across samples from diverse tumor types.

Methods

Comprehensive tumor and immune profiling with the ImmunoID NeXT Platform®

To address the challenge of providing characterization of both the tumor and TME, we have developed the ImmunoID NeXT Platform, an augmented, immuno-oncology-optimized exome/transcriptome platform designed to provide comprehensive information from a single FFPE tumor sample.

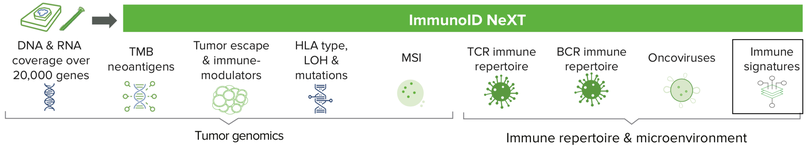

Figure 1: Overview of results provided in ImmunoID NeXT.

Development of an immune infiltration quantification methodology

To generate our reference data, we profiled the transcriptomes of eight purified immune cell types using the ImmunoID NeXT Platform. We verified the utility of the transcriptome profiling results by comparing to published gene sets[2] representing some of the immune cell types. Then, we utilized these transcriptome profiles to develop platform-specific reference expression signatures specific for each cell type. We also evaluated a selection of published gene sets, and selected between our in-house and published gene sets based on internally-developed criteria. Finally, we used ssGSEA[3], which provides a semi-quantitative score that can be compared across samples for the same cell type, to score the abundance of each immune cell type.

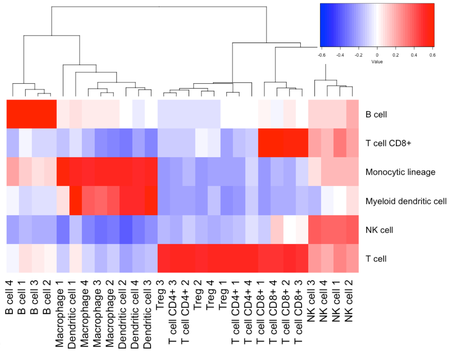

Figure 2: Heatmap of ssGSEA scores for purified immune cells using published gene sets.

Results

Quantification concordance with immune cell mixtures

To first demonstrate accuracy of our quantification methodology, we created mixtures combining four purified immune cell populations at different ratios. We then compared the quantifications from our transcriptome-based approach to the immune cell fractions measured by flow cytometry. The strong concordance suggests that our scores accurately reflect the underlying immune cell composition.

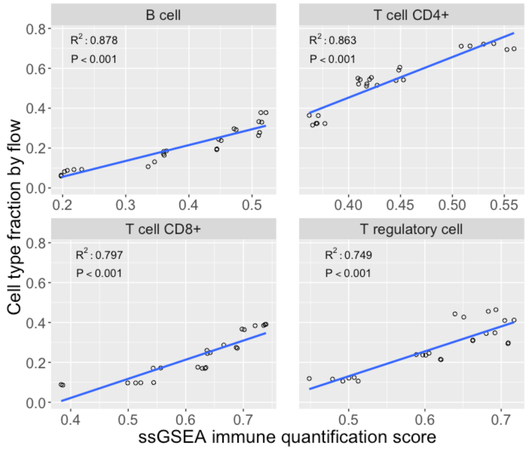

Figure 3: Comparison of ssGSEA scores using the ImmunoID NeXT Platform with immune cell fractions evaluated with flow cytometry for immune cell mixtures.

Quantification concordance with healthy PBMC samples

To further demonstrate the accuracy of our methodology, we performed both transcriptome sequencing and cytometry by time of flight (CyTOF) on healthy donor PBMCs. We observe a significant relationship between our immune quantification scores and the abundances from CyTOF. This suggests accuracy at immune profiling in real samples with diverse immune populations.

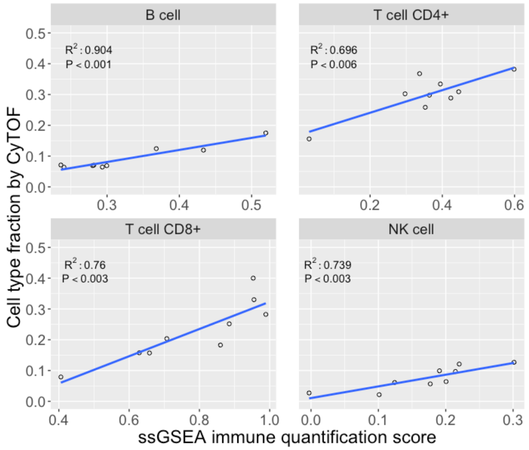

Figure 4: Comparison of ssGSEA scores using the ImmunoID NeXT Platform with immune cell fractions evaluated with CyTOF for healthy donor PBMCs.

Profiling of immune infiltration across tumor types

Finally, we highlight the diversity of immune populations across cancer types by applying the ImmunoID NeXT Platform to 433 tumor samples from 13 different tumor types. This is part of an ongoing effort to profile a diverse set of tumor types with the ImmunoID NeXT Platform, which will provide a deeper understanding of the distributions of many immune features, including quantification of immune cell types. Additional information on this study can be seen on our companion poster, #2512.

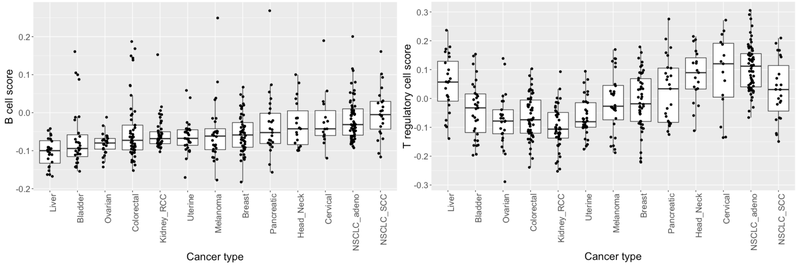

Figure 5: Comparison of ssGSEA scores representing B cells and T regulatory cells across 433 tumor samples from 13 tumor types.

Conclusion

Analysis of the immune infiltrate of tumor samples can add to our understanding of the tumor-immune interaction, with potential applications including studies of response to immunotherapy. RNA sequencing can be utilized as a scalable approach for such analysis. Here, we test the accuracy of our approach using multiple sample sets with orthogonal profiling. We demonstrate that the ImmunoID NeXT Platform can accurately evaluate the composition of infiltrating immune cells in tumor samples.

References

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