The immune expression landscape of solid tumors with tertiary lymphoid structures

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Introduction

- Tertiary lymphoid structures (TLS) are lymphoid organs that develop in non-lymphoid structures in chronically inflamed areas such as tumors. These organized aggregates of T and B cells have the potential to be used as prognostic and predictive cancer factors and could even be targeted for activation and differentiation to increase the antitumor immune response.
- One current challenge is to identify TLS biomarkers that can discriminate between tumors with strong immune infiltration and those with true TLS structures. **In this study, we identified genes and pathways that could potentially be used to categorize tumors as TLS-positive.**

Methods

- Targeted RNA-seq of 398 immune genes was performed on 167 FFPE tumors representing 5 histologies, composed of melanoma (51.5%), lung cancer (29.9%), kidney cancer (16.2%), bladder cancer (1.8%), and head and neck cancer (0.6%).

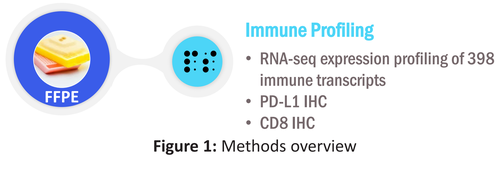


Figure 1: Methods overview

- These tumors had different degrees of T-cell immune infiltration patterns as determined by H&E and CD8 IHC review: TLSs (14.4%), strongly infiltrating (7.2%), infiltrating apparent (29.9%), infiltrating not apparent (22.2%), and not infiltrating (26.3%).
- We then identified differentially expressed genes comparing TLS-positive tumors with TLS-negative tumors (including infiltration patterns), and TLS-positive tumors with strongly infiltrated tumors, followed by pathway analysis with the REACTOME database.

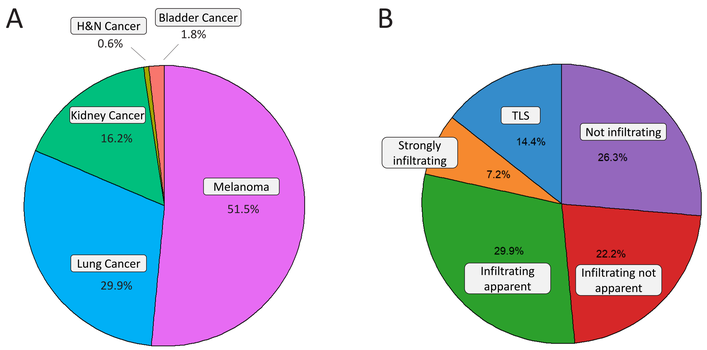


Figure 2: 167 samples from clinically tested FFPE tumors spanning (A) 5 cancer types and (B) 5 categories of immune infiltration. Cohort primarily consists of melanoma followed by lung cancer and kidney cancer. Inclusion criteria for the samples was based on clinical QC parameters for RNA-seq.

Results

When comparing TLS-positive with TLS-negative tumors, TLS-positive tumors were significantly enriched in pathways associated with the adaptive immune system and T cell receptor signaling, whereas TLS-negative tumors were enriched in cytokine and interferon signaling (Figure 3).

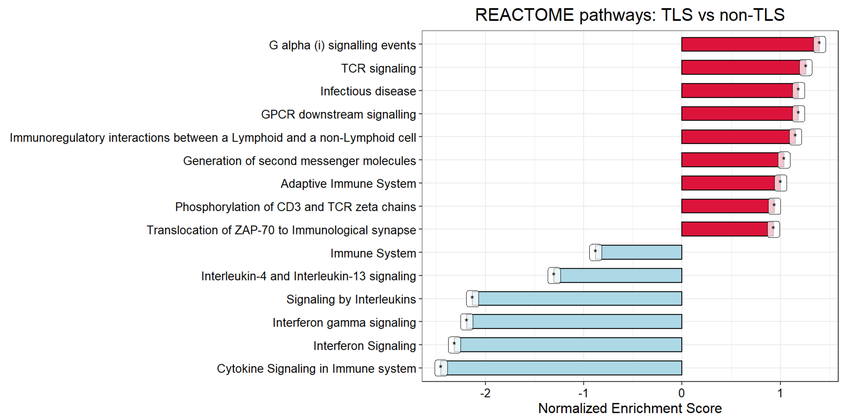


Figure 3: Gene Set Enrichment Analysis with REACTOME pathways database for tertiary lymphoid structures (TLS) vs all other non-TLS categories. Red bar is for upregulation and blue bar for downregulation. * = p value <0.05.

When comparing TLS-positive tumors with strongly infiltrated tumors, we observed that TLS-positive tumors were significantly enriched in pathways associated with cell cycle, mitosis, and proliferation signaling hinting at active antigen recognition and anti-tumor response (Figure 4).

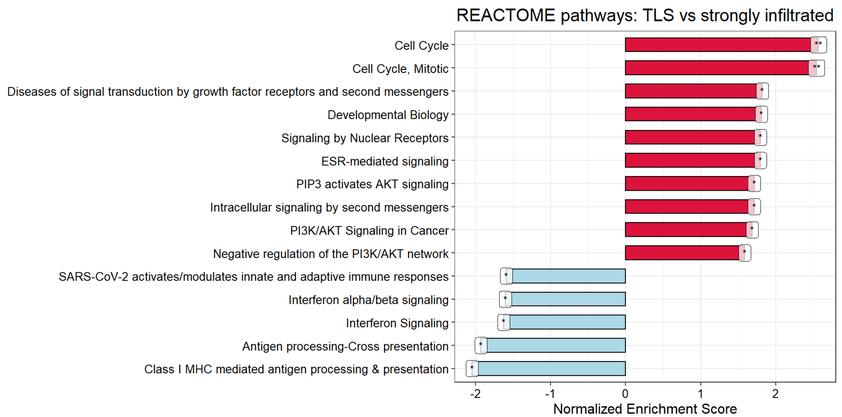
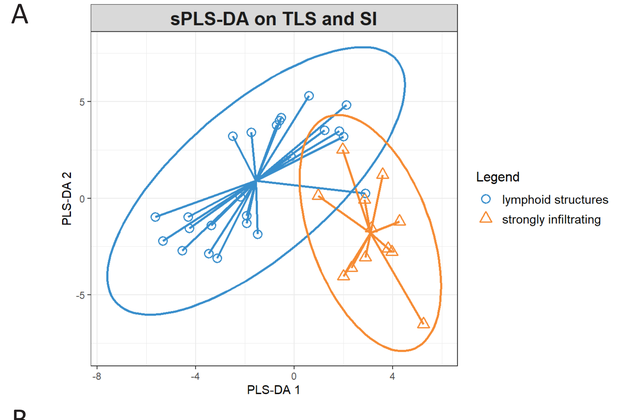


Figure 4: Gene Set Enrichment Analysis with REACTOME pathways database for tertiary lymphoid structures (TLS) vs strongly infiltrated tumors. Red bar is for upregulation and blue bar for downregulation. * = p value <0.05, ** = p value <0.01.

We then trained a sparse partial least squares discriminant analysis (sPLS-DA) classification model using gene expression data from TLS and strongly infiltrated (SI) tumors to identify the top genes differentially expressed between these groups (Figure 5). Among the top contributors for component 1 were (for TLS) genes EGR3, TUBB and MYC, associated with cell cycle and structural components, and (for SI) genes LRG1, OAS1 and IFI27, associated with immune response.



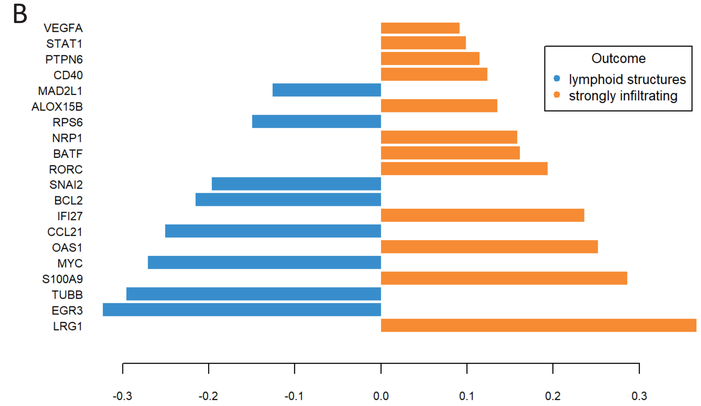


Figure 5: Sparse partial least squares discriminant analysis (sPLS-DA) classification model on gene expression data of TLS and strongly infiltrated tumors. (A) Samples projected into the space spanned by the first two components including 95% confidence ellipses. (B) Top 20 contributors (genes) to component 1.

Conclusions

Our results suggest that tumors with TLS have an upregulation of pathways associated with cell cycle and proliferation, and downregulation of interferon and cytokine signaling pathways compared to strongly immune infiltrated tumors without TLS. Genes associated with these pathways could be potential biomarkers for use in identifying TLS-positive tumors. Future directions are to continue the refinement of these signatures using larger datasets and explore their value in predicting clinical outcomes in patients treated with immune checkpoint therapy.

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