Elucidating cell-cell interactions in pediatric and adolescent Hodgkin Lymphoma for targeted therapy by single cell RNA sequencing

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Introduction

Due to continuous development of Hodgkin Lymphoma (HL) therapy treatment over the last few decades, the overall survival rates of children and young adult patients currently exceed 90%. However, further improvement of treatment is needed to reduce the severe long-term treatment-associated toxicity, such as infertility, cardiovascular complications and high rates of novel second cancers.

Classical HL is characterized by a low frequency (~0.1-5%) of the malignant Hodgkin Reed-Sternberg (HRS) cells, while non-malignant T and B lymphocytes are the predominant cell type in the tumor microenvironment (TME). The presence of these immune cells is probably essential for HRS cell survival. HRS-TME interactions have been studied previously, with PD1-PDL1 and CTLA4-CD80/CD86 as some of the most extensively studied interactions. Most studies applied immunohistochemistry staining and cell culture experiments, which only partially capture the interactions. Here, we aim to study all interactions that are present *in vivo* on a per-tumor basis by applying single cell RNA-sequencing (scRNA-seq) to pediatric and adolescent classical HL samples.

Methods

For each tumor biopsy, we sorted live cells to get an unbiased overview of the TME and use a flow cytometry antibody panel to enrich for HRS cells. HRS marker expression as determined by diagnostic immunohistochemistry was compared to the scRNA-seq expression profiles to identify tumor cells. We determined the single-cell copy-number status from the expression data to confirm the presence of tumor cells. Immune cell types were determined by previously published methods and marker gene expression.

Results

The HRS cells in our dataset express PDL1, CD80, and CD86. Few T cells express the corresponding receptor PD1 but most expressed CTLA4. In one sample, no HRS cells could be detected, likely due to sampling the healthy counterpart of the diseased lymph node. Interestingly, a subset of T cells of this sample expressed high levels of PD1, which is in correspondence with previous findings. In addition, this was the only sample that contained a significant number of germinal center B cells, indicating clear differences in the microenvironment near HRS cells. Finally, we applied published methods to study cell-cell interactions in scRNA-seq data.

Conclusion

We applied single cell RNA sequencing to molecularly characterize the Hodgkin lymphoma microenvironment and idenitify therapeutic targets, thereby increasing our understanding of this complex disease.