Single-cell RNA sequencing analysis of thyroid nodule fine needle biopsy aspiration

Caitlin Bell, MD[1], Maria Albuja-Cruz, MD[2], Mohammed Alshalalfa, PhD[3], Brian Cervenka, MD[4], Kristin Gleitsman, PhD[3], Joshua P. Klopper, MD[3], Chris Raeburn, MD[2], Andrew Uzilov, PhD[3], Bryan Haugen, MD[1], Nikita Pozdeyev, MD[1,2]

1. University of Colorado Anschutz Medical Campus, Division of Endocrinology, Diabetes, and Metabolism; 2. University of Colorado Anschutz Medical Campus, Department of Surgery; 3. Veracyte, Inc.; 4. University of Colorado Anschutz Medical Campus, Department of Otolaryngology; 5. University of Colorado Anschutz Medical Campus, Department of Biomedical Informatics

University of Colorado Anschutz Medical Campus

veracyte

OBJECTIVE

- Molecular testing with bulk RNA and DNA sequencing is used for thyroid nodule fine needle aspiration (FNA) biopsies with indeterminate cytology. While the sensitivity of these tests is excellent, specificity and prognostic information could be improved.
- Single cell RNA sequencing (scRNAseq) is a novel approach used to identify cell subpopulations in tumor microenvironments and has the potential to identify diagnostic and prognostic molecular data in cell clusters not identified in bulk sequencing.
- There is scarce data on the diagnostic potential for scRNAseq for thyroid cancer, especially from preoperative samples.
- **We sought to determine the feasibility of using scRNA seq on thyroid nodule FNA samples.**

METHODS

Adults referred for thyroid nodule FNA biopsy or undergoing thyroidectomy were enrolled in the study

↓

FNA biopsies were performed under US guidance for a total of 3-4 FNA passes per nodule

↓

Material was collected for both bulk RNA seq (Afirma GSC) and scRNAseq in separate tubes

↓

Digestion and RBC lysis were optimized for scRNAseq with >70% cell viability and capture of follicular-derived cells

↓

Bioinformatics analysis was done using standard scRNAseq data analysis pipeline in Seurat package in R

RESULTS

Enrollment Data

- Total of 18 subjects were enrolled → 16 completed scRNAseq → 10 had high quality data (presence of follicular cells)
- Average age was 54.8 years
- 6 women and 4 men
- 4 biopsies were performed in clinic; 6 in OR (prior to neck incision)

ScRNAseq Data:

Cytology or surgical pathology diagnosis	N	Avg # total cells	Avg # follicular cells
Benign	5	4054	84
PTC (ATA low risk of recurrence (ROR))	3	3176	109
PTC (ATA high/intermediate ROR)	2	7809	1039

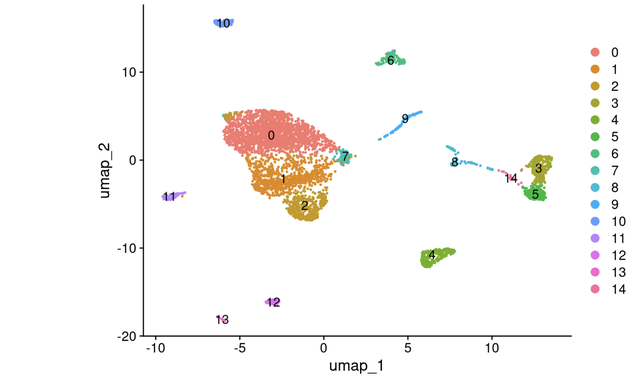

Figure 1. Example of scRNAseq from FNA sample. Majority of clusters (0,1,2,4,7,8,9) are immune cells, likely from peripheral blood. Cluster 6 and 10 are follicular cells.

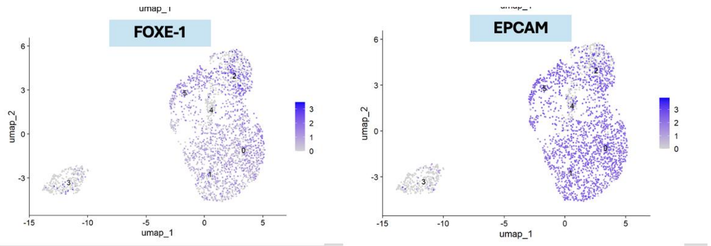

Figure 2. Reclustering follicular cells with high quality samples (n=7). Two distinct clusters of follicular cells are identified; de-differentiation is seen in the smaller cluster.

RESULTS (CONT.)

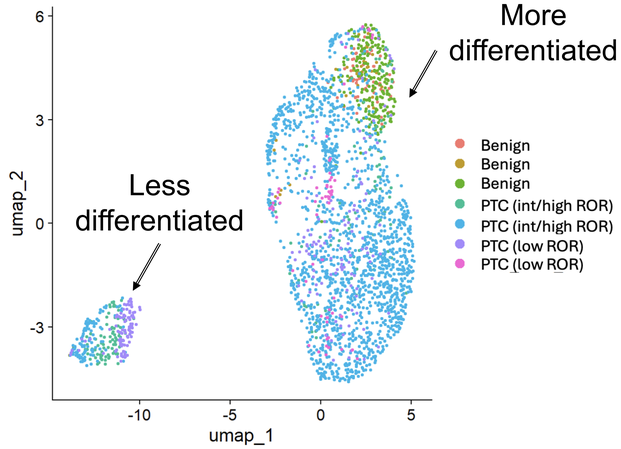

Figure 3. Identifying follicular cell clusters. The less differentiated cluster contains cells from both intermediate/high risk for PTC ROR (blue and teal) and one low risk tumor (purple)

CONCLUSIONS

- We developed a protocol that demonstrates the feasibility of scRNAseq on thyroid nodule FNA samples.
- Our scRNAseq analysis showed that most cells from FNA are immune cells, likely from peripheral blood. Importantly, we were able to differentiate benign and malignant samples by follicular cell clustering.
- Furthermore, our scRNAseq analysis showed that more aggressive cancers seem to demonstrate unique clusters with less differentiated cells, which could enhance FNA molecular prognosis.
- Future studies will evaluate the concordance of scRNAseq from different cell populations with the Afirma GSC bulk RNAseq results.