Epithelial-to-mesenchymal transition (EMT) regulation revealed by the single-cell RNA-seq analysis in melanoma

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Introduction

Single-cell RNA sequencing (scRNA-seq) is a revolutionary technology that allows you to evaluate the transcriptome of many individual cells in a sample.

This approach has become essential to advance understanding of the molecular mechanisms that regulate tumor progression and prospect new eligible targets for developing prognostic or therapeutic biomarkers.

Long non-coding RNAs (lnc-RNAs) have been reported to play essential roles in regulating the hallmarks of cancer capabilities (Ref. 1), including the activating invasion and metastasis, which are considered critical hallmarks responsible for around 90% of cancer deaths (Ref. 2).

The epithelial-mesenchymal transition (EMT) is a vital mechanism underlying metastasis in cancer patients. Understanding the role of lncRNAs in the regulation of EMT may shed light on the mechanism of metastasis activation and lead to new approaches to cancer treatment.

Material and Methods

We exploit public RNA-seq data from single melanoma cells to study gene pathways regulating metastasis (Ref. 3, Fig. 2). We use appropriate algorithms to identify subpopulations, cell types, enriched pathways, and trajectories (Fig. 1).

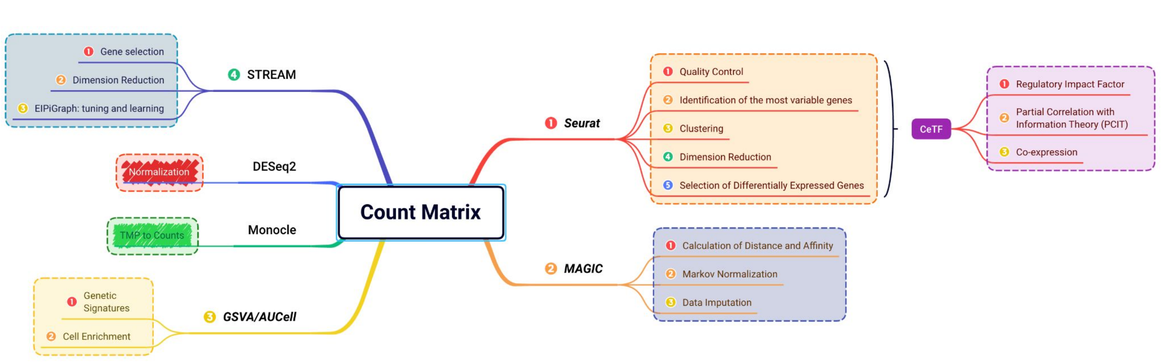

Figure 1. Workflow of methods used in this work include Seurat → CeTF, MAGIC, GSVA/AUCell, and STREAM tools.

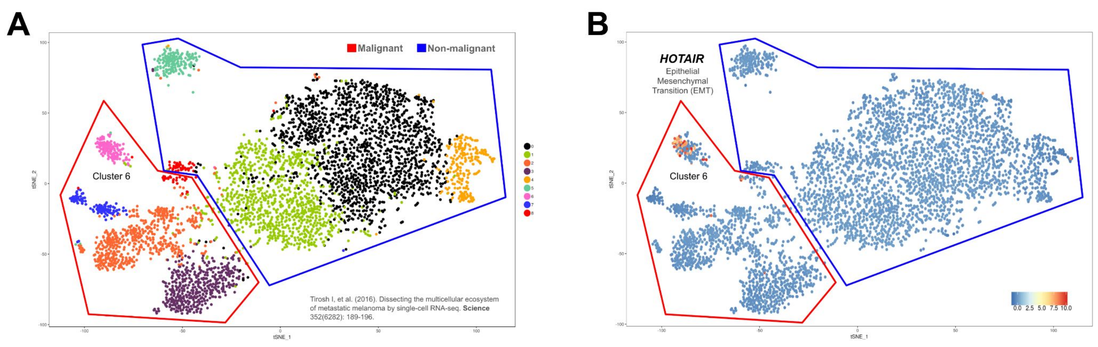

Figure 2. Nonlinear dimensionality reduction Approach. **A**) Unsupervised clustering for analyzing all malignant and non-malignant melanoma cells characterized in nine cluster cells. **B**) Cluster cell number 6 shows overexpression of lncRNA Hotair, characterizing it as a putative metastatic cell.

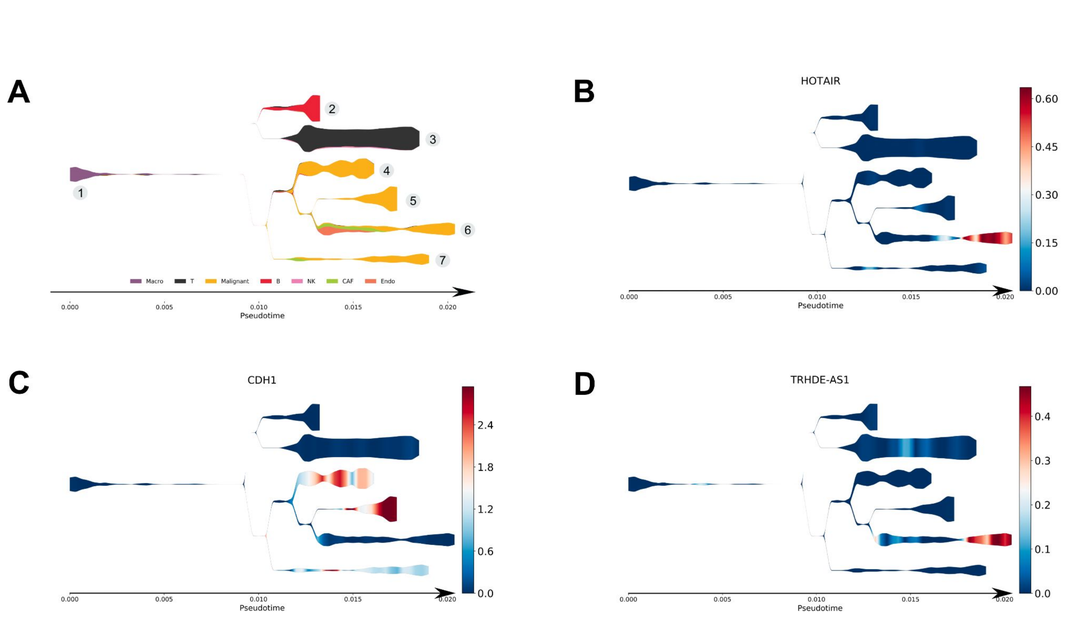

Figure 3. Trajectory inference in melanoma scRNA-seq. **A)** Flow chart performed by the STREAM inferring the trajectory of seven cell types in melanoma; **B)** Group of cells expressing the *HOTAIR* located at the end of arm 7; **C)** *CDH1* does not co-express with HOTAIR, suggesting EMT activation; **D)** TRHDE co-expressing with HOTAIR, suggesting its participation in the regulation of the EMT.

Results

Preliminary results suggest that lncRNA TRHDE-AS1 could act by regulating the EMT pathway in partnership with HOTAIR.

Also, it was possible to propose a gene circuit of the negative feedback type, where HOTAIR positively regulates TRHDE-AS1, and TRHDE-AS1, upon reaching a certain level of expression, negatively regulates HOTAIR.

Single-cell trajectory analysis showed the possible point of activation of the EMT mechanism by CAF cells. These findings reinforce the potential of the scRNA-seq approach in better understanding cancer biology.

Conclusion

Putting all results together, we present reliable data that suggest a negative feedback mechanism between two lncRNA (HOTAIR and TRHDE-AS1) regulating EMT.

The next step is to identify the protein complexes that interact with HOTAIR and TRHDE-AS1 to clarify how both lncRNAs cooperate in regulating EMT in melanoma.

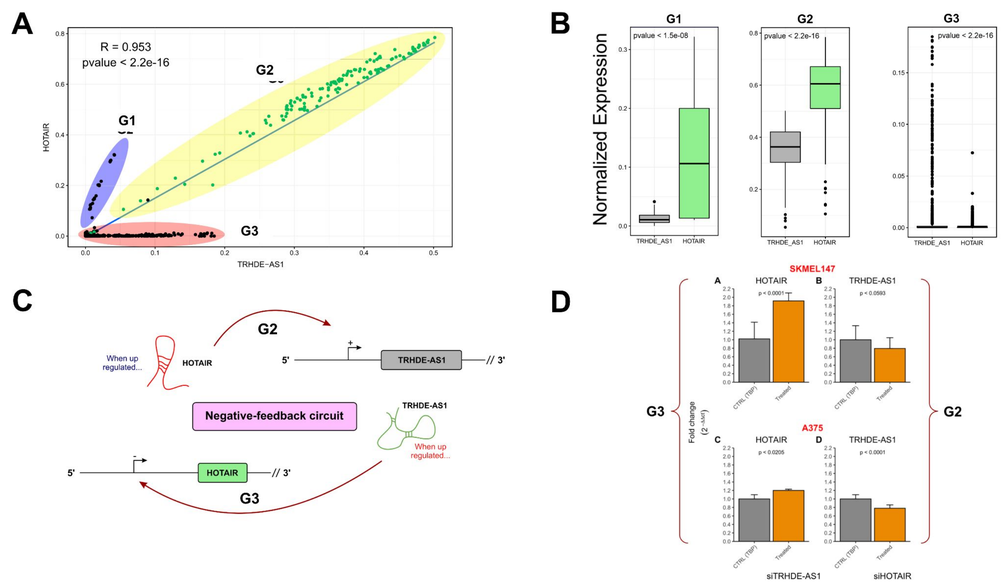

Figure 4. Preliminary validation of the negative feedback circuit (NFC) involving HOTAIR and TRHDE-AS1 co-regulation in EMT activation. **A and B**) Correlation graph between the expression of the TRHDE-AS1 (axis x) and HOTAIR (axis y) genes. The subclusters are broken down into C1, C2 and C3, with a correlation of 0.953 between the cells coexpressing HOTAIR and TRHDE-AS1; **C**) Negative-feedback circuit proposed to regulating EMT in melanoma cells; **D**) Functional validation of Negative feedback circuit where HOTAIR upregulates TRHDE-AS1, and TRHDE-AS1 downregulates HOTAIR in two melanoma cell lines.

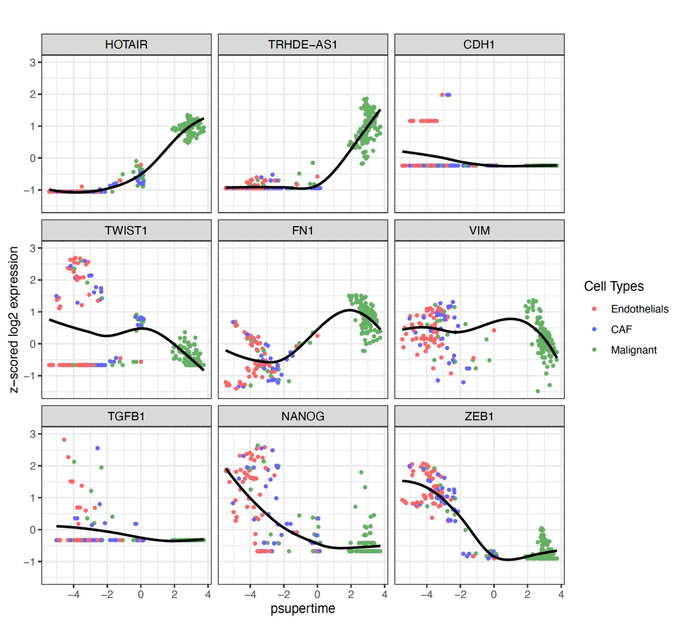

Figure 5. Pseudotime was calculated using the pseudotime tool showing the pseudotime value on the x-axis and the z-score expression in log2 on the y-axis. The dots with different colors represent the three cell types (endothelial, CAF, and malignant) present in cluster 6 (Fig. 2b). The genes represented in these figures are related to the EMT pathway..