

# Identification of Metastatic Cells in Undifferentiated Pleomorphic Sarcoma: Single Cell RNA Sequencing and CIBERSORT Analysis

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## INTRODUCTION:

Undifferentiated Pleomorphic Sarcoma (UPS) is one of the most common types of malignant soft tissue sarcoma. It has a high recurrence rate of around 40% and a discouraging prognosis, with a 5-year survival rate of 50-60%. Lung metastasis accounts for 90% of distant metastasis in UPS, suggesting that preventing lung metastasis could improve patient outcomes. Research on tumor heterogeneity, the diverse characteristics of cells within a tumor, has been growing. In UPS metastasis, this heterogeneity also has received attention, and our research lab has contributed by discovering the coexistence of metastatic and non-metastatic cells in the same tumor. This study aims to identify and understand these elusive metastatic cells in UPS, enhancing our knowledge and potentially leading to innovative treatments.

## METHODS:

We collected five samples of primary tumors diagnosed as UPS. Among the collected samples, one exhibited lung metastasis at the time of sampling. Single-cell RNA sequencing (scRNA-seq) was performed on the collected samples, and clustering was conducted after integration. We detected distinctive clusters associated with the samples exhibiting lung metastasis and identified feature genes specific to those clusters. Gene Set Enrichment Analysis (GSEA) was also performed using the feature genes. Furthermore, we obtained bulk expression data of UPS from The Cancer Genome Atlas and analyzed the presence of clusters in the results of scRNA-seq for each sample using the analysis tool called CIBERSORT. By comparing tumors with lung metastasis to tumors without lung metastasis, we validated the distinctive clusters associated with tumors that had lung metastasis.

## RESULTS:

After filtering the five samples based on sample quality, we were able to analyze 3777, 6629, 4664, 7074, and 6060 cells in each respective sample. Integration was performed based on canonical correlation analysis, followed by a shared nearest neighbor modularity optimization-based clustering, resulting in the identification of 13 clusters. Among these clusters, three clusters (cluster #5, #6 and #12) were found to be more abundant in the samples from primary tumors with lung metastasis compared to the ones without lung metastasis (Figure A). Furthermore, according to the results obtained from CIBERSORT analysis, cluster #12 was relatively abundant in tumors with lung metastasis (Figure B). Analyzing the feature genes of cluster #12 based on these findings revealed an upregulation in the expression of genes such as BRINP3, CDH12, and EPHA7. To elucidate the functions of these feature genes, GSEA was performed, revealing a significant negative enrichment score for Epithelial-Mesenchymal Transition (Normalized enrichment score = -2.89, adjusted p-value < 0.001). These findings suggest an association of Mesenchymal-Epithelial Transition (MET) with lung metastasis.

## DISCUSSION:

By conducting analysis using scRNA-seq and CIBERSORT, we were able to identify genes as well as gene sets that characterize metastatic cells in UPS. Our result showed that MET related to lung metastasis, which is consistent with reports describing hybrid epithelial/mesenchymal phenotype is associated with metastasis. The further functional evaluation of feature genes, both in vitro and in vivo, is a future task to determine how they contribute to metastatic ability.

## SIGNIFICANCE/CLINICAL RELEVANCE:

This study, which explored the characteristics of cells that metastasize within the UPS, could contribute to the elucidation of metastasis mechanisms.

