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Integrated Bulk and Single-Cell Transcriptomics Identify Liver-Adaptive Tumor States in Metastatic siNET

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BACKGROUND

Liver metastasis is a major determinant of poor outcome in small-intestinal neuroendocrine tumors

(siNETs), yet mechanisms enabling metastatic adaptation remain unclear. Whether siNET liver

metastases simply reflect neuroendocrine (NE) tumor cells growing in hepatic tissue or include tumorassociated

cells with liver-adaptive transcriptional programs remains unknown. We hypothesized that

siNET liver metastases contain compartmentalized tumor states, including remodeled NE cells and liveradaptive

hepatocyte (hep)-like cells.

METHODS

Public bulk RNA-seq from GSE98894 was analyzed by differential expression and pathway enrichment

across primary and liver-metastatic siNETs. To validate these findings at single-cell resolution, we

analyzed five matched primary/metastatic siNET pairs using in-house scRNA-seq integrated with

Seurat/Harmony. Cell states were annotated using curated NE, hepatic, epithelial, stromal, and immune

markers. NE and hep-like clusters were analyzed by marker expression, module scoring, differential

expression, enrichment analysis, and Slingshot trajectory inference. Unresolved cells were refined into

expanded NE and hep-like compartments. Patient-aware pseudobulk differential expression,

inferCNV/CNV-like burden scoring, and DecontX ambient RNA correction assessed remodeling, tumor

association, and liver RNA contamination. A single-cell-derived NE/liver-adaptive/epithelial signature

was evaluated in 72 bulk siNET samples from GSE98894.

RESULTS

Bulk RNA-seq showed that siNET liver metastases upregulated liver/metabolic programs compared with

primary tumors. Single-cell clustering revealed distinct NE and hep-like populations within the metastatic

niche. NE clusters retained CHGA, CHGB, SYP, INSM1, PCSK1, and TPH1, whereas hep-like clusters

were enriched for TTR, APOA1, ALB, and fibrinogen genes. Pseudotime suggested remodeling across

primary- and metastasis-associated NE and hep-like states. Patient-aware pseudobulk identified 264

significant genes between metastatic and primary expanded NE cells and 139 between metastatic

expanded hep-like and metastatic expanded NE cells. Expanded hep-like and NE compartments showed

comparable inferCNV-derived CNV-like burden, higher than other cells, supporting tumor association of

a subset of hep-like cells. DecontX correction did not abolish the liver-adaptive program. In bulk

validation, the liver-adaptive score distinguished liver metastases from primary siNETs (FDR=7.85e-9;

AUC=0.922), and a hybrid score was also enriched in metastasis (FDR=8.72e-4; AUC=0.748).

CONCLUSIONS

Integrated bulk and single-cell transcriptomics support a model in which siNET liver metastases contain

remodeled NE compartments and a candidate tumor-associated liver-adaptive hep-like state. Defining

these compartment-specific states may nominate metastasis-associated programs for future therapeutic

targeting. Tissue-level validation is warranted to confirm NE, liver-adaptive, and epithelial marker coexpression

in metastatic tumor cells.

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