

Integrative Spatial Transcriptomics of Copper Driven Pathway Activity and TME Signaling in Colon Cancer

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ABSTRACT

- Colon cancer is the third most common cancer in the world and is a leading cause of cancer deaths
- Copper accumulation progresses the growth of colon cancer, but there is limited knowledge on its spatial distribution and how it affects gene expression and signaling within the TME
- Application of ST aids in categorizing molecules to determine the functional organization of the TME

INTRODUCTION

- **Colon Cancer & Copper:** Colon cancer is a leading cause of cancer death. Copper (Cu) is known to accumulate in tumors and drive cancer progression, making it a compelling therapeutic target
- **Gap:** While copper's role has been studied and established, knowledge on its spatial distribution within the tumor microenvironment (TME) and its local effects on signaling are limited
- **Spatial Transcriptomics:** Integrative spatial transcriptomics allows the mapping of gene expression within the intact tissue architecture, revealing the functional geography of the TME
- **Hypothesis:** We hypothesize that copper-driven processes are not random but are organized into functional niches within the colon cancer TME, and that this spatial organization influences local pathway activation
- **Approach:** Application of spatial transcriptomics to a colon cancer sample to map cuproptosis and copper metabolism pathways, aiming to quantify pathway activity and the key genes designing them and to better understand their spatial niches

METHODS

- **Spatial Transcriptomics:** A human colon cancer sample was profiled using the 10X Genomics Visium platform to obtain spatially resolved gene expression data.
- **Gene Selection:** A core set of 12 genes involved in copper metabolism and cuproptosis was selected from a genetic analysis of a pan-cancer study
- **Pathway Definition:** Genes were grouped into three functional pathways
 - Copper Transport: SLC31A1 (import), ATP7A, ATP7B (export)
 - Mitochondrial Copper Handling: SLC25A3, COX17, SCO1, SCO2, COX11, COX19
 - Cuproptosis: FDX1, DLAT, LIAS
- **Pathway Scoring:** Activity scores for each pathway were calculated for every spatial spot using the `sc.it.score_genes()`, which compares pathway gene expression to a reference set
- **Integrative Analysis:** Spearman's correlation was used to identify the dominant copper gene proxy most predictive of each pathway's activity
- **Spatial Statistics:** Moran's I was applied to quantify the spatial clustering of pathway scores and confirm non-random organization

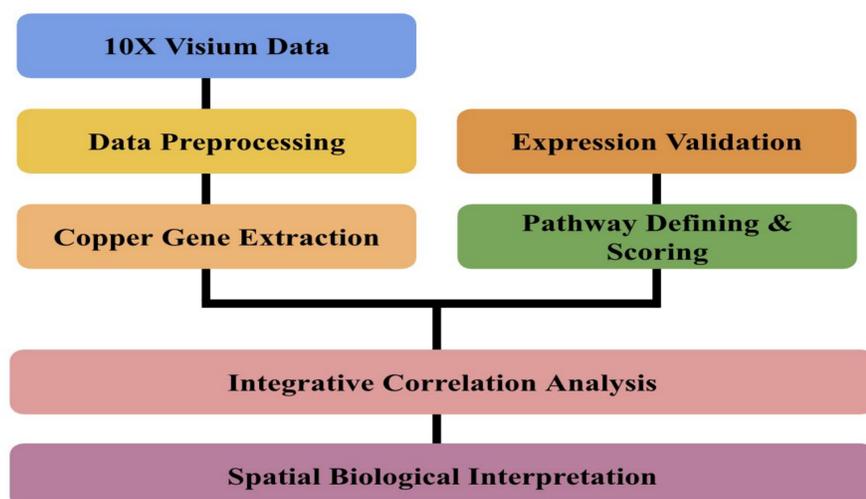


Figure 1. Methodology Workflow

RESULTS

Figure 2. Spatial distribution and correlation analysis of copper-related pathway scores.

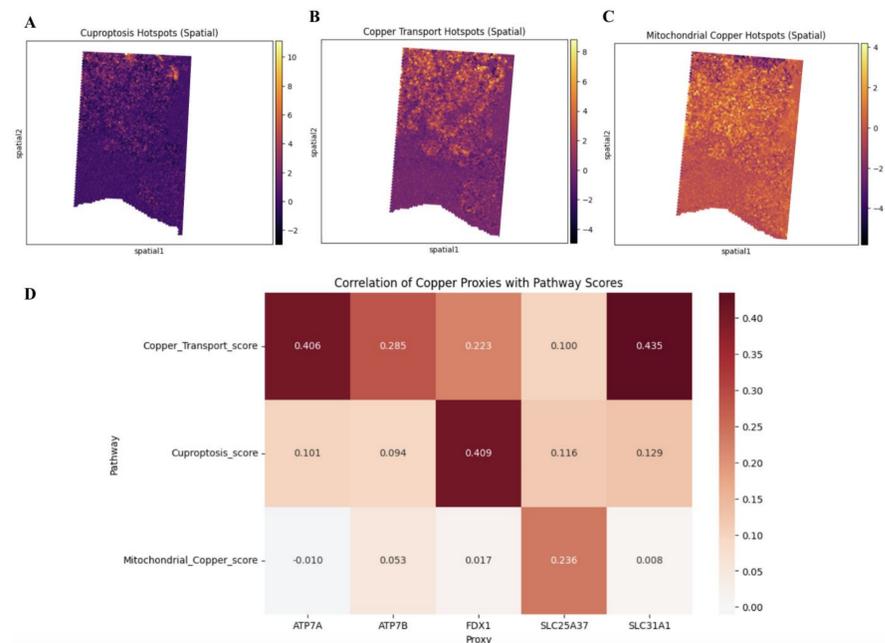


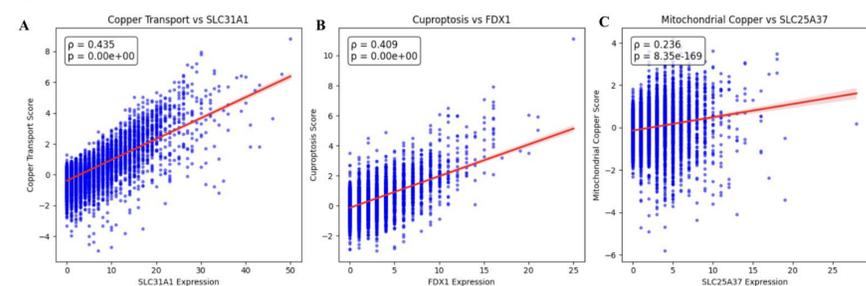
Table 1. Quantification of spatial organization with spatial autocorrelation analysis using Moran's I

Pathway	Moran's I	p-value	Spatial Clustering
Copper Transport	0.444	<0.05	Yes
Mitochondrial Copper	0.293	<0.05	Yes
Cuproptosis	0.248	<0.05	Yes

Table 2. Spearman correlation results of Cu related proxy with pathway scores to identify dominant mechanism for each pathway

Pathway	Top Correlate	Spearman's ρ	p-value (threshold)
Copper Transport	SLC31A1	0.435	< 0.001
Mitochondrial Copper	SLC25A3	0.236	< 0.001
Cuproptosis	FDX1	0.409	< 0.001

Figure 3. Regression plots of pathway and dominant proxy relationships



Pairwise relationships between the dominant copper proxy gene and its corresponding pathway score for all spots in the sample

CONCLUSION

- **Potential for Clinical Impact:**
 - Refines our understanding of copper biology in colon cancer from a spatial context
 - Findings provide a framework for developing targeted therapeutic strategies, such as selectively increasing copper levels in FDX1-high regions or starving aggressive tumor niches of copper, offering new insights for precision medicine in colorectal oncology.
- **Limitations:**
 - Gene expression was used as a proxy for copper metal distribution as opposed to integrating spatial metabolics or elemental mapping techniques due to limited time
- **Future Work:**
 - Implement other techniques for mapping copper
 - Validate findings across more patients and colon cancer cancer subtypes
 - Use ML models (GNNs) to predict copper and other transition metal-driven pathways and outcomes
- **Data and Code Availability:**
 - Data/code available on reasonable request, privacy/ethical restrictions.

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References: Available using QR code:

