Altered Copper Metabolism in Granuloma Annulare
Revealed by Spatial Transcriptomic Profiling

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Background

- While altered copper metabolism is a known host defense mechanism against mycobacterial infection, the role of copper metabolism in non-infectious granulomas is unknown[1-5]
- We examined differential expression of copper homeostasis genes (CHGs) with spatial transcriptomics (ST) in granuloma annulare (GA) skin biopsy samples

Methods

- Skin punch biopsies were prospectively collected from affected and control skin of 6 patients with clinical and histologic diagnosis of GA
- Visium10x protocol was used for ST analysis
- Distinct cell clusters were identified using gene ontology analysis (Figure 1)

Table 1. Expression levels of copper homeostasis genes in patient skin biopsies

Gene Cell cluster	GA affected	Healthy control	P-value
SLC31A1			
GA microenvironment	0.4303*	0.1091*	0.00011
STEAP4			
GA microenvironment	0.39059*	0.1461*	0.04686
ATOX1			
GA granulomatous center	1.064*	0.5499*	0.00135
GA microenvironment	0.9343*	0.195*	0.00002
IL2RG			
GA granulomatous center	0.54*	0.1797*	0.00030
GA microenvironment	0.4079*	0.05742*	0.00017

*Normalized mean expression of the gene of interest across 6 patient samples.
SLC31A1 encodes the main copper influx transporter protein CTR1
STEAP4 encodes a metalloreductase of copper ions to allow for cellular copper uptake
ATOX1 encodes a copper metallochaperone protein
IL2RG encodes the cytokine receptor gamma subunit responsive to IL-2 and other interleukins

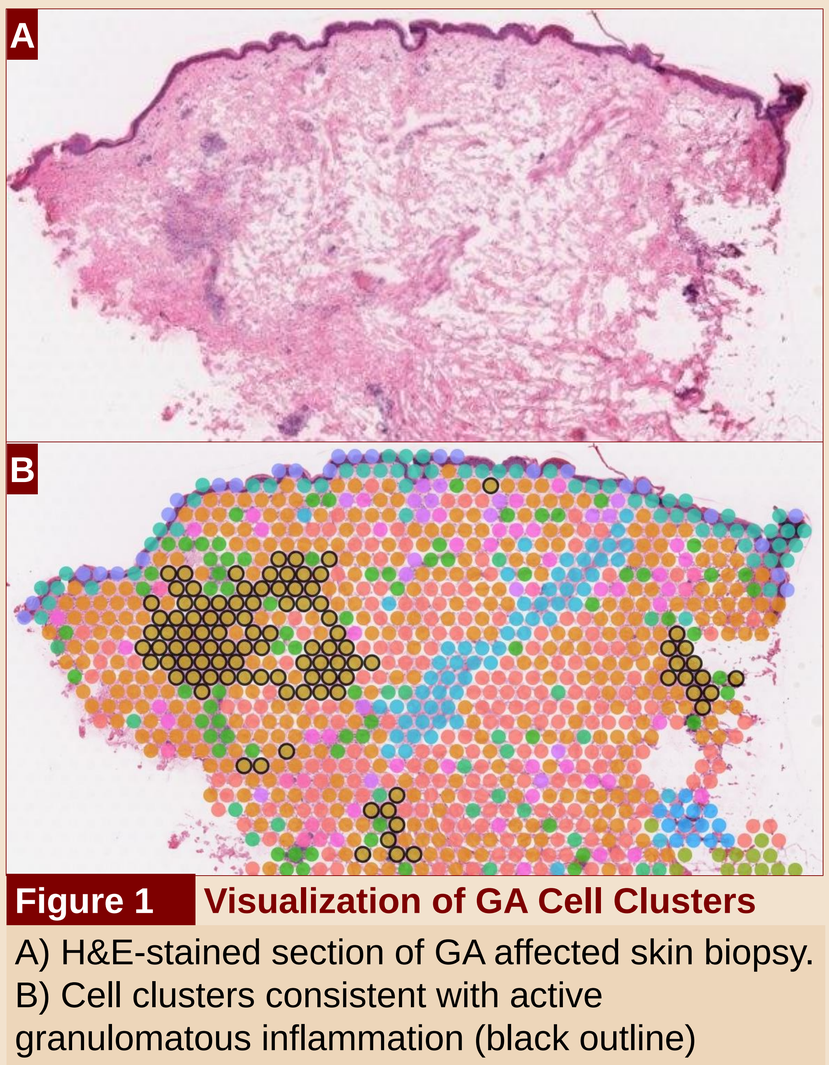

Figure 1 **Visualization of GA Cell Clusters**

A) H&E-stained section of GA affected skin biopsy.
B) Cell clusters consistent with active granulomatous inflammation (black outline) mapped onto the tissue section.

Results

- Compared with contralateral healthy control skin samples, ST analysis of the affected GA skin samples showed differential expression of several CHGs:
  - Solute carrier family 31 member 1 (SLC31A1)
  - Six transmembrane epithelial antigen of prostate 4 (STEAP4)
  - Antioxidant 1 copper chaperone (ATOX1)
  - Cytokine receptor common subunit gamma (IL2RG)
- Summary of gene expression levels provided in Table 1.

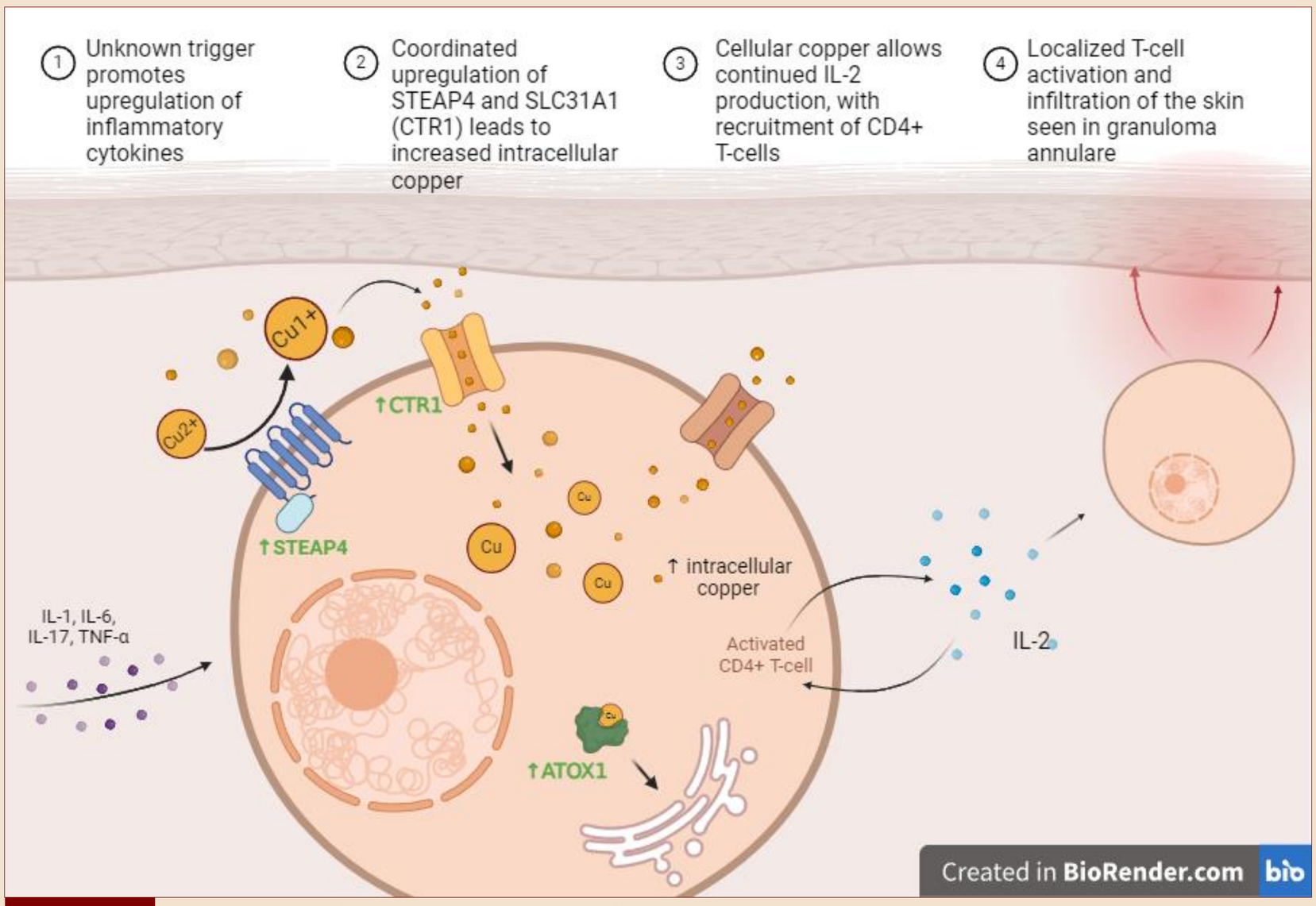

Figure 2 **Proposed Role of Copper Metabolism in GA Pathogenesis**

We hypothesize that inflammatory markers triggers coordinated upregulation of copper homeostasis genes, including STEAP4, SLC31A1, and ATOX1. Sufficient cellular copper levels allow for continued production of inflammatory cytokines that lead to activation and recruitment of CD4+ T-lymphocytes. Infiltration of the skin by activated T-cells lead to clinical manifestations of granuloma annulare.

Discussion

- We hypothesize that inflammatory signals in local APCs lead to disrupted copper metabolism in adjacent tissue.
- Compared with controls, ST analysis revealed increased expression of inflammatory mediators and CHGs mapped to GA granulomatous inflammation and the GA microenvironment cell clusters.
- Our ST findings support closer examination of the role of copper and copper metabolism in GA pathogenesis.

Affiliations and References