Single-cell transcriptomics reveals cellular heterogeneity and phenotypic transitions of smooth muscle cells in aortic dissection

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Liang Shao, Fan Hu, Lingna Zhao, Jianping Luo, Pengtao Zou, Xiu Liu, Shaoyi Zheng, et al., Single-cell transcriptomics reveals cellular heterogeneity and phenotypic transitions of smooth muscle cells in aortic dissection. ***iMeta*** 5: e70124.
https://doi.org/10.1002/imt2.70124

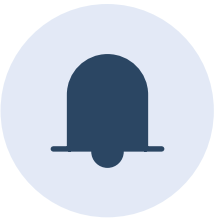
Highlights

1. First Definition of Two Key Pathological Types of Smooth Muscle Cells (SMC) Subtypes

Fiberized SMC (SMC2)

Core characteristics: Strong epithelial-mesenchymal transformation (EMT) tendency, which dominates extracellular matrix (ECM) remodeling.

Mechanism: Upregulation of collagen genes (COL1A1/3A1) and stromal cytokines (CCN2), directly accelerating the fibrosis process.



Inflammatory SMC (SMC3)

Core characteristics: presenting typical hypoxic microenvironmental adaptation characteristics, strongly driven by inflammatory signals.

Mechanism: Activation of MAPK signaling pathway, secretion of chemokines (CXCL10, etc.), recruitment of immune cells to exacerbate inflammation.

Key insights: Two subtypes jointly drive aortic dissection pathology through different mechanisms

2. Revealing the association of thrombosis formation with the immunosuppressive microenvironmen

Key Findings

• In thrombotic-positive AD samples, angiogenesis-type 4 (ANGPTL4) was significantly upregulated
• Significantly increased proportion of M2-type macrophages with immunosuppressive function

Potential Mechanisms

• Thrombotic regions may form a hypoxic, stressful microenvironment
• This microenvironment induces immunosuppressive states, which in turn affect disease progression and tissue repair

Highlights

3. Discovery of Key Signals for Vascular Homostasis Disbalance —VEGFA Signaling Source Transference

Normal State

Signal source: smooth muscle cells
VEGFA signals are mainly sent by SMCs to maintain basic communication.

Core Function: Maintaining homeostasis
Ensures vascular structure integrity and suppresses abnormal transparency.

Pathological State

Signal source: fibroblasts
The signaling body undergoes metastasis and is no longer dominated by SMCs.

Pathological consequences: homeostatic imbalance
Disruption of vascular communication order, causing pathological generation and leakage.

Core mechanism: The transfer of VEGFA signaling sources from SMCs to fibroblasts is a key inducer of vascular homeostasis imbalance

Highlights

4. Validation of key findings through in vitro functional experiments

SMC2 Induces Endothelial Transformation

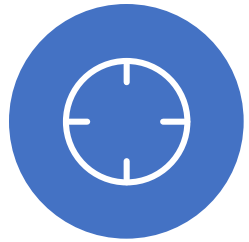
Experimental method: Building a co-culture system of SMC2 with endothelial cells to simulate microenvironmental interactions

Experimental Results: Endothelial cells were successfully induced to undergo mesenchymal transformation (EndMT) and their reproductive ability was significantly inhibited.

SMC3 Regulates Inflammatory Pathways

Experimental method: Conditioned medium treatment of monocytes is collected, and cell chemization and activation states are analyzed

Experimental results: Significantly promoted monocyte migration and secreted a large amount of inflammatory mediators through activation of the MAPK pathway.



Validation of Key Protein Expression

Experimental method: Immunofluorescence technology was used to colorate and localize AD tissue samples.

Experimental Results: Intuitive confirmation that key proteins such as IGFBP2, PLOD2, and VEGFA are upregulated in expression in tissues.

Conclusion: Multidimensional in vitro experiments (cell co-culture/chemical experiments/immunofluorescence) provide solid functional evidence support for sequencing findings

Background

Aortic dissection (AD): A severe cardiovascular challenge

Definition and Pathophysiological Mechanisms
AD is a fatal acute condition characterized by a tear in the inner membrane of the aortic artery, with blood seeping into the middle layer to form a false cavity. The core mechanism is the weakening of the middle layer structure, and high blood pressure is the main trigger factor.

Critical Disease: Immediate Intervention
Medical or surgical intervention must be performed immediately to prevent fatal complications such as aortic rupture and cardiac congestion.

Severe status quo: high mortality
Despite medical technology advancements, AD mortality rates remain high, highlighting the urgent need to deeply understand its pathogenesis.

Core insights: Early identification and mechanism research are key to reducing mortality

Background

Limitations of Existing Research and Purpose of This Study: Exploring from Risk Factors to Mechanisms

Accumulation of Known Risk Factors

- Genetic predisposition and family history
- High blood pressure and vascular damage
- Influence of connective tissue diseases

Multi-factor overlap leads to pathological susceptibility

Pathological Mechanism Understanding Blind Zones

- The cellular and molecular mechanisms driving the development of AD development are not fully elucidated
- Limited understanding of the specific roles and interactions of different cell types

Cell interactions and molecular pathways to be analyzed

Core Purpose

✓Identifying Key SMC Subtypes: Unveiling Core Role Subgroups

✓Clarifying Mechanical Role: Analyzing Fibrosis and Inflammatory Driving

✓Exploring Cell Interactions: Building Communication Network Maps

Revealing Mechanisms for SMC Subtype-Driven AD Pathology

Methods

01 Research Overview

Subject study: aortic dissection (AD) Depth sequencing based on surgical resection of lesion tissue in 15 patients

Strict sample grouping strategy

• AD group: 10 cases (3 positive for thrombosis / 7 negative for thrombosis)

• Control group: 5 cases (adjacent normal aortic tissue)

Single Cell Capture Scale

145,660 cells were successfully captured and transcriptome analysis completed

02 In Silico Analysis

Cell Clustering and Annotation | UMAP Visualization Accurately Identifies 9 Major Cell Types

Differential expression analysis | Comparing gene expression differences between different groups, locking markers

Functional Enrichment Analysis | GSEA Enrichment Analysis, Deeply Reveals Cell Functional Characteristics

Cell Trajectory Analysis | Pseudo-time analysis to infer SMC phenotypic transformation path

Intercellular Communication Analysis | CellChat Constructs Signal Networks to Analyze Interaction Mechanisms

03 Wet Lab

Cell Culture and Induction

In vitro induced SMC exhibits fibrosis or inflammatory phenotype

Co-cultivation with Transwell

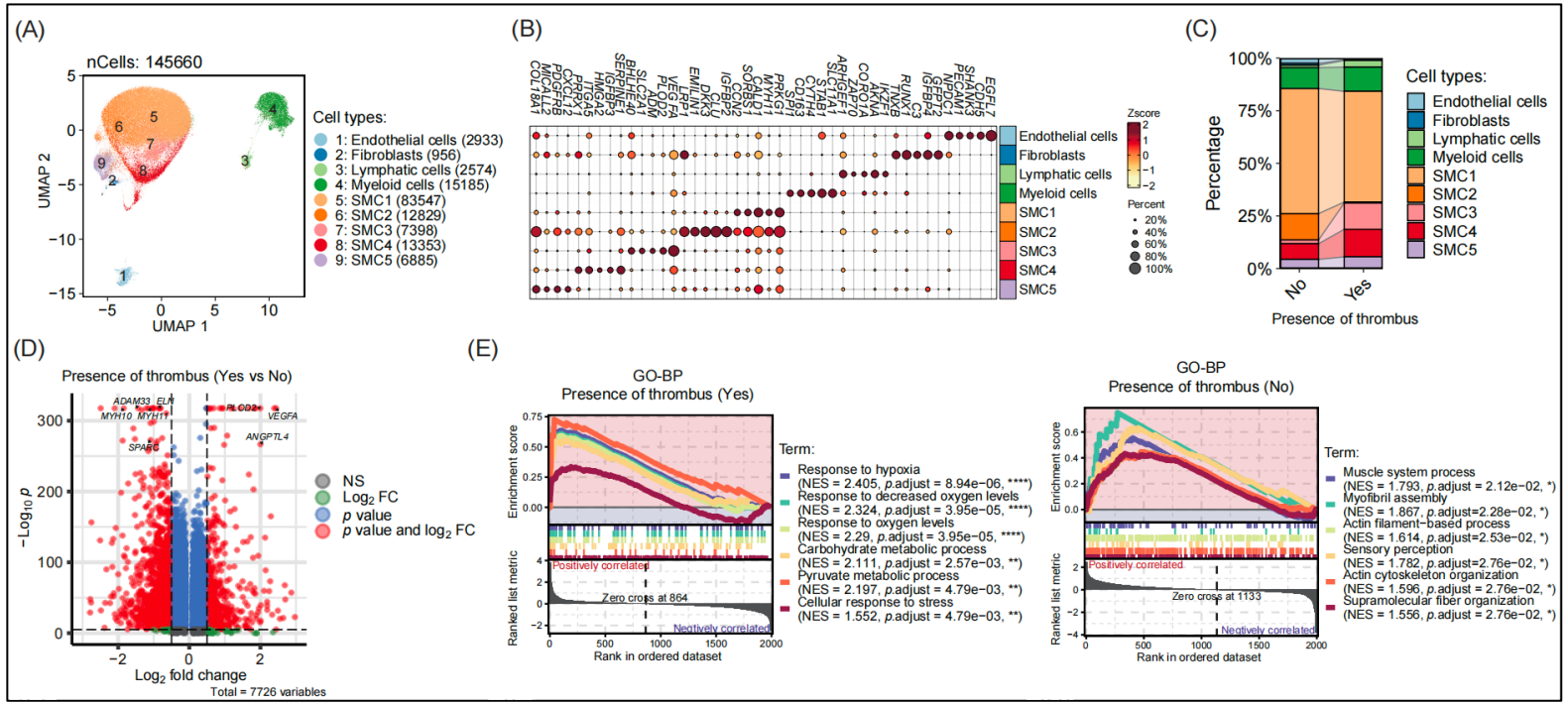
Building a co-culture system to validate cell migration and interaction

Key Protein and Pathway Validation

Western Blot and the Core Pathways for Immune Fluorescence Detection

Results

Single-cell RNA sequencing reveals cellular heterogeneity in thrombus-related aortic dissection



Key Findings

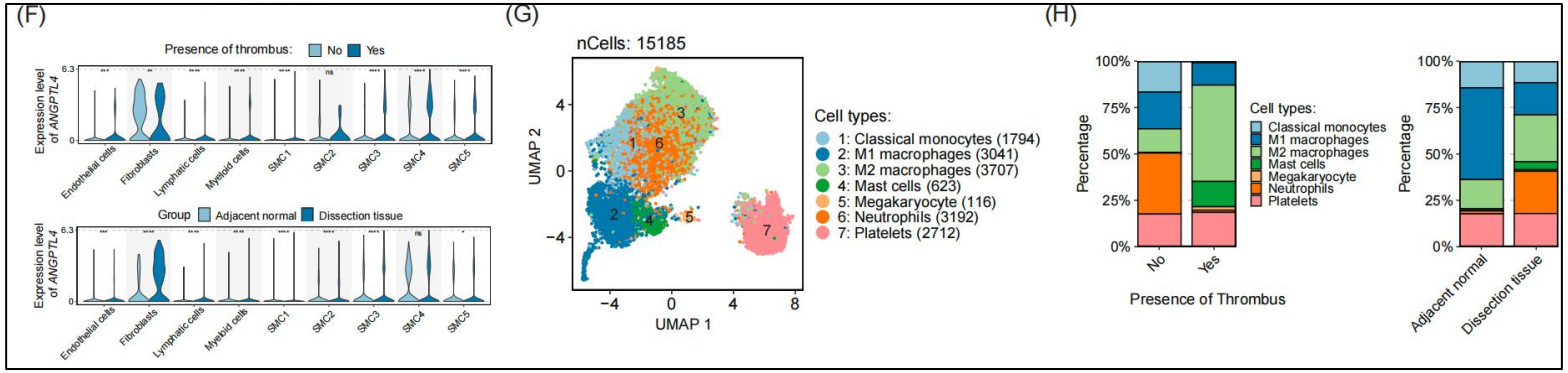
Panorama Cell Typing Identification

UMAP maps clearly show nine major cell types, constructing a panoramic picture of cell heterogeneity in AD tissues.

Research conclusion: Single-cell transcriptome sequencing technology provides a high-resolution cellular perspective for analyzing the pathogenesis of thrombotic-related aortic dissection.

Results

Single-cell RNA sequencing reveals cellular heterogeneity in thrombus-related aortic dissection



Key Findings

Significant differences in key genes

Comparing thrombosis-positive/negative samples, genes such as ANGPTL4 were found to be significantly upregulated, suggesting their potential role in thrombosis.

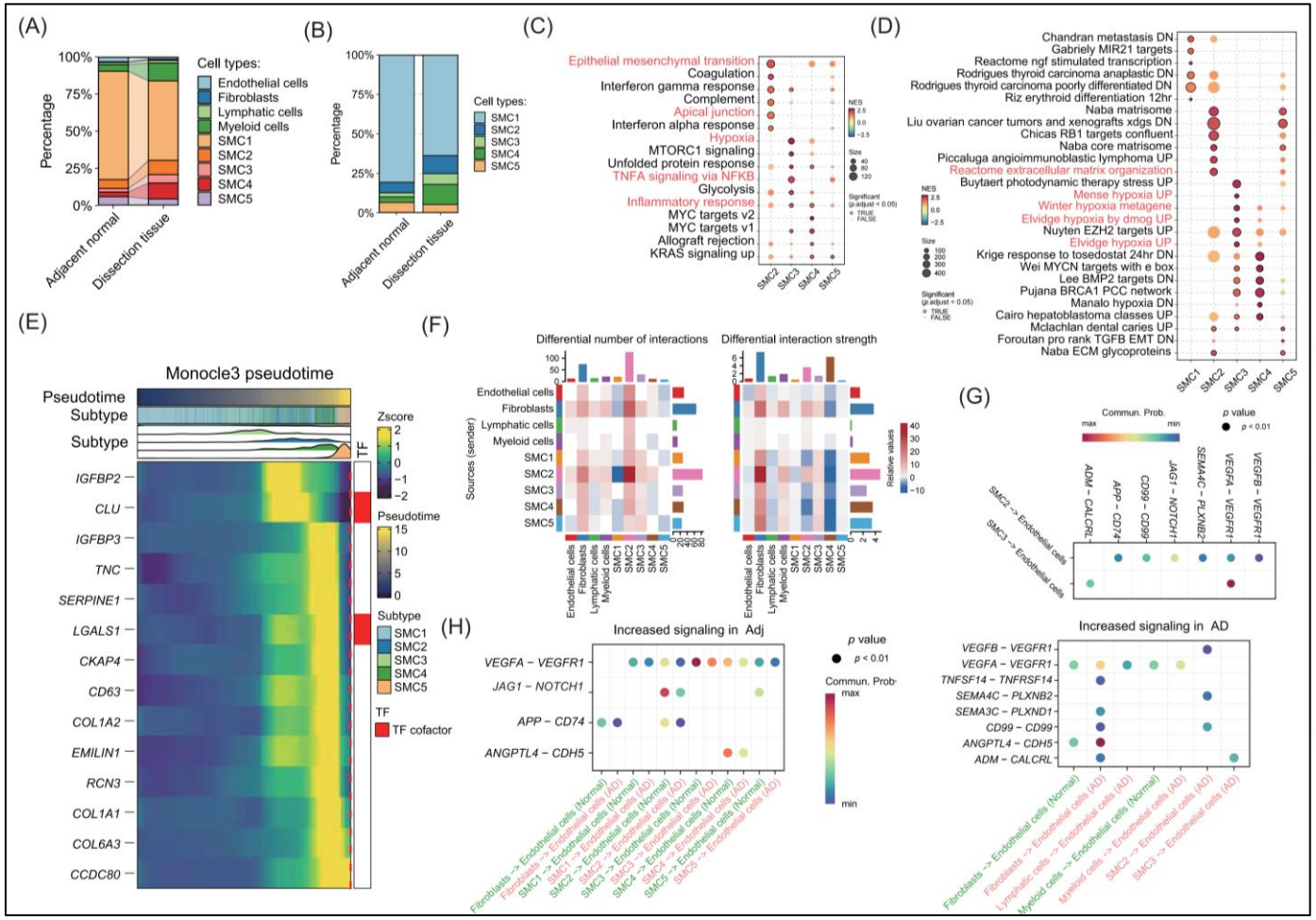
Functional pathways and immune characteristics

Enrichment of hypoxia/stress pathways, and M2-type macrophages exhibit specific enrichment trends in AD lesion tissues.

Research conclusion: Single-cell transcriptome sequencing technology provides a high-resolution cellular perspective for analyzing the pathogenesis of thrombotic-related aortic dissection.

Results

Identification and characterization of smooth muscle cell subpopulations in Aortic Dissection



Accurate Identification of 5 SMC Subtypes

SMC2 with fibrosis characteristics and SMC3 with inflammatory characteristics were successfully distinguished, revealing key differences in cell heterogeneity in AD tissues.

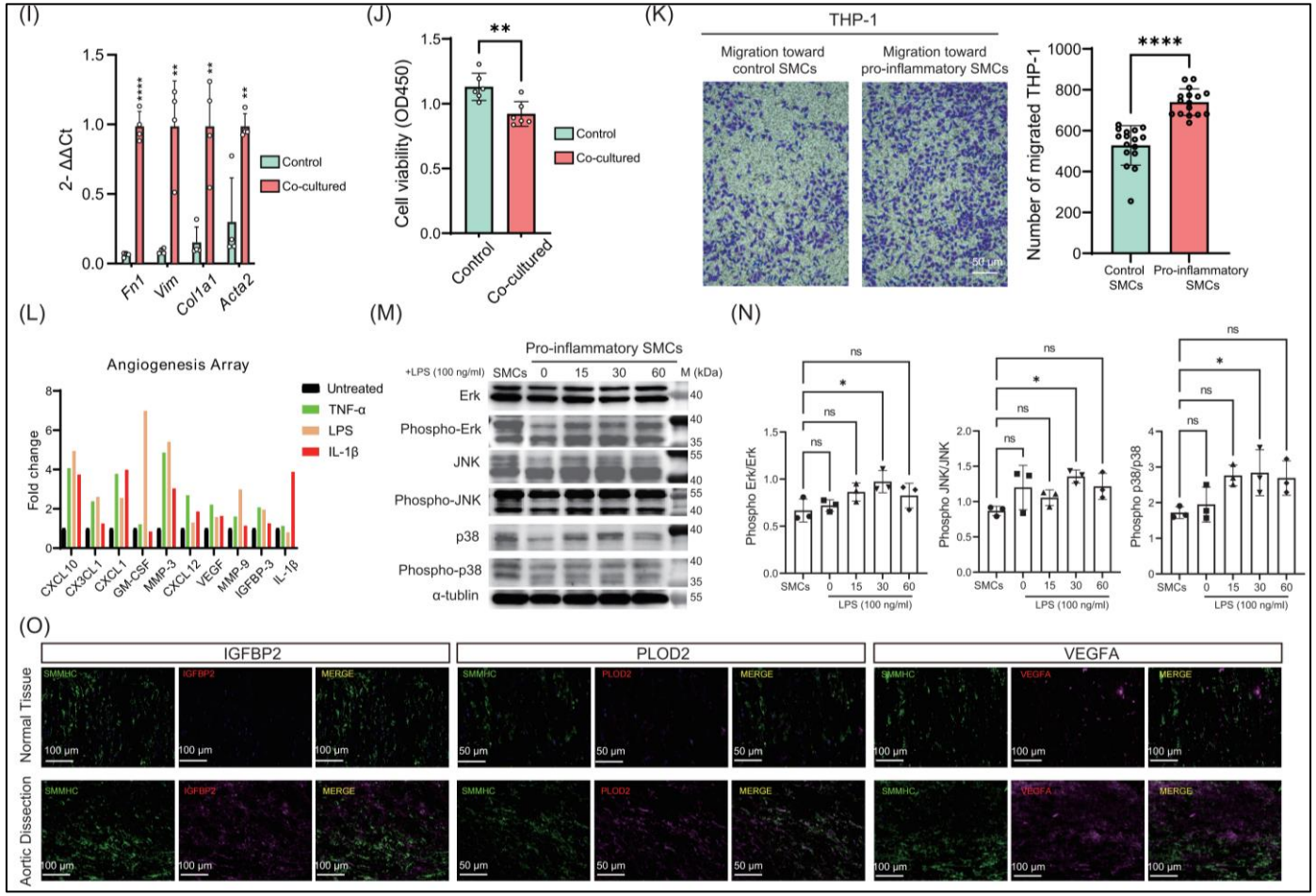
Dynamic transformation and communication enhancement

Pseudo-time analysis depicted the cell transformation path; intercellular communication analysis confirmed a significant enhancement in AD tissue signaling and metastasis of the VEGFA signal source.

Core Conclusion: Heterogeneous remodeling of smooth muscle cells is the key mechanism for aortic dissection occurrence

Results

Identification and characterization of smooth muscle cell subpopulations in Aortic Dissection



Multivariate validation in vitro

The core functions of SMC subtypes in AD pathology were validated in all directions, from cell proliferation and migration ability to the activation state of key signaling pathways.

Core Conclusion: Heterogeneous remodeling of smooth muscle cells is the key mechanism for aortic dissection occurrence

Heterogeneity and functional phenotypic analysis of SMCs

SMC2：Fibrotic Phenotype

Functional characteristics: EMT and ECM remodeling

It exhibits strong epithelial mesenchymal transformation (EMT) characteristics and dominates abnormal sedimentation and remodeling of extracellular matrix (ECM), which is the core driver of tissue fibrosis.

Key markers: collagen vs. CCN2

Collagen synthesis genes COL1A1, COL3A1, and stromal cell derivative factor CCN2 were significantly upregulated.

SMC3：Inflammatory/Hypoxic Phenotype

Functional characteristics: hypoxia and inflammation drive

Duplicately regulated by microenvironmental hypoxia and chronic inflammation signals, metabolic reprogramming occurs, showing high sensitivity to damage stress.

Key markers: contraction downregulation/metabolic upregulation

Contraction markers MYL9, ACTA2 were downregulated; hypoxia/glucose fermentation genes PLOD2, PFKFB3 were significantly upregulated.

Core conclusion: SMC subtype functional differentiation is significant in AD tissues, and fibrosis and inflammatory hypoxia are two key phenotypic characteristics.

Key Findings

Reconstructing and Functional Validation of Intercellular Communication Networks

01 Remodeling

Communication Enhancement: The number and intensity of intercellular interactions in AD tissues significantly increase, and cell-cell “dialogues” become unusually frequent.

Key signal transfer: The VEGFA signal source is converted from SMCs to fibroblasts, directly disrupting the homeostasis of the blood vessels.

02 Validation

EndMT Induction

Fibrotic SMC significantly inhibits endothelial cell proliferation

Inflammatory Driven

Extremely Significant Increase in Single-Nucleus Cell Migration Capability

MAPK pathway activation

ERK, JNK, p38 factors are in a highly activated state

Conclusion

Specific SMC subtype drive pathology

The study clarified that SMC2, which promotes fibrosis, and SMC3, which promotes inflammation, are core cell subtypes driving AD pathological processes.

Thrombosis is associated with immunosuppression

The thrombosis region may build an immunosuppressive microenvironment, which may contribute to further worsening of the disease.

Cell communication network imbalance

The abnormal transfer of VEGFA signals from SMCs to fibroblasts is a key inducer of vascular homeostasis disruption.

Revealing Potential Treatment Targets

Key SMC subtypes, MAPK pathways, and key molecules (ANGPTL4, VEGFA, etc.) have been proven to be highly potential targets for drug intervention.

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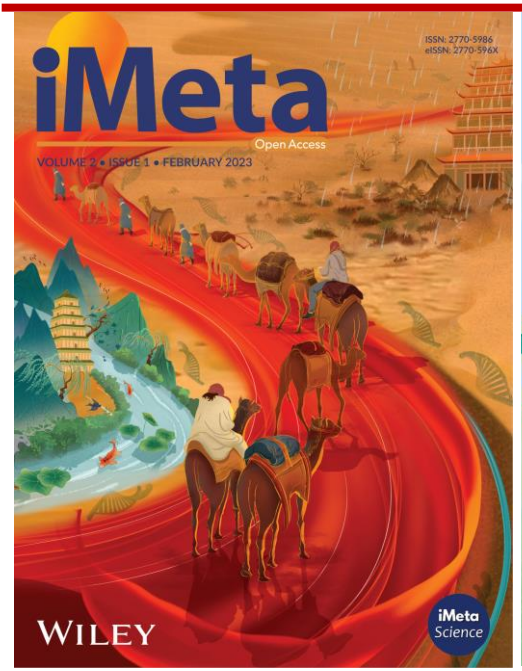

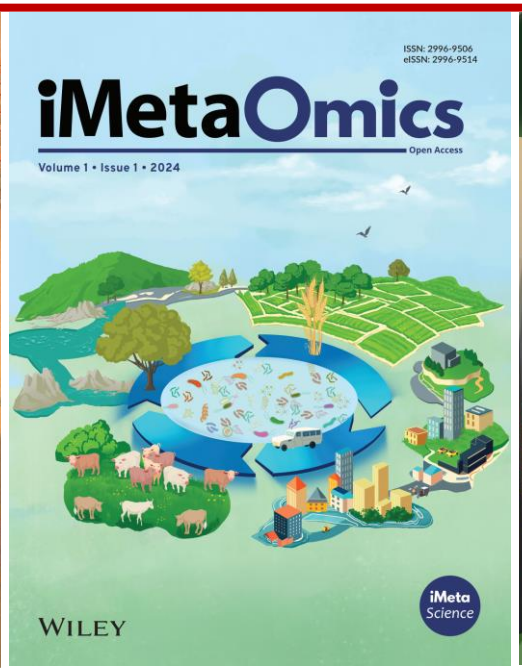

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