Beyond the Pump: Identifying Neuron-Like Cells and Chimerism in Cardiac Tissue

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Abstract

Unexpected emotional and behavioral changes following heart transplantation inspired this study to investigate whether neuron-like cells might exist within the heart. To explore this question, single-cell and single-nucleus RNA sequencing data from zebrafish, mouse, and human heart tissues were analyzed. Raw data from zebrafish and human samples were processed using Cell Ranger, while preprocessed mouse nuclei data were integrated directly.

Analysis revealed neuron-like clusters expressing enriched neuronal and neurotransmitter-related gene signatures, suggesting the presence of cells with neural-like transcriptional profiles across species. In parallel, human heart transplant data were examined using the SNP-based tool Souporcell to assess chimerism. This analysis confirmed the persistence of donor-derived cells within the recipient heart.

Together, these results highlight an opportunity to explore how cellular integration in transplanted organs may intersect with underexplored aspects of memory, emotion, or identity.

Keywords

Neuron-like Cells, Chimerism Analysis, Single-cell RNA Sequencing, Single-nucleus RNA Sequencing, Zebrafish Heart, Human Heart

Introduction

Is the heart simply a pumping machine, or is there more to it? Traditionally, the heart has been viewed as a mechanical organ responsible for circulating blood. However, several unusual stories from heart transplant recipients have sparked scientific curiosity [1]. In one case, a child who loved Power Rangers began rejecting them after receiving the heart of another child who died retrieving a Power Ranger toy. Another developed an unexplained fear of water, similar to the circumstances of their donor's drowning. A third recipient described sensations of burning and flashes of light on his face, echoing the fatal injury of his donor, a police officer who was shot.

Though anecdotal, such cases raise an intriguing question: could the heart contain cells that influence memory, emotion, or aspects of personal identity?

To explore this question from a molecular perspective, single-cell and single-nucleus RNA sequencing data from zebrafish, mouse, and human heart tissues were analyzed. Zebrafish were selected due to their transparent heart, rapid development, and conserved gene pathways relevant to cardiac and neural biology. Mouse heart nuclei were included to assess the presence of similar transcriptional patterns in mammals. Human heart transplant data were used to evaluate donor-recipient chimerism through genotype-free clustering with Souporcell.

Through this cross-species transcriptomic approach, the analysis aims to identify neuron-like gene expression in cardiac tissue and to determine whether donor-derived cells persist in transplanted human hearts. These findings contribute to broader discussions on how organ transplantation may intersect with memory, emotion, or personal identity.

Methods

1. Data Sources

This study analyzed three distinct datasets:

Zebrafish: Single-cell RNA-seq including whole heart and atrium samples, obtained from a public GEO dataset [2].

Mouse: Single-nucleus RNA-seq dataset from 10x Genomics (5k Mouse Heart Nuclei) [3].

Human: Single-nucleus RNA-seq from a heart transplant sample (left ventricle) with cardiac allograft vasculopathy [4].

2. Data Input and Preparation

Zebrafish samples were imported using `sc.read_10x_mtx()` from Cell Ranger output. The atrium and whole-heart matrices were labeled and merged using common genes. The mouse dataset was downloaded as a filtered `.h5` file and read using `sc.read_10x_h5()`. The human dataset was analyzed from a filtered file created by Cell Ranger.

3. Quality Control and Filtering

Filtering thresholds were adapted per dataset:

Zebrafish: Cells with <200 or >5000 genes and >5% mitochondrial content were excluded. Doublets were removed with Scrublet.

Mouse: Cells with 100–3500 genes were retained, reflecting the lower complexity of nuclear RNA. Mitochondrial filtering was skipped. Scrublet was applied.

Human: No filtering or doublet removal was applied; the dataset was used as processed by Cell Ranger.

4. Normalization and Batch Correction

Raw counts were passed directly to scVI, which performs internal normalization, scaling, and batch correction. The scVI latent space was used for all downstream clustering, visualization, and gene expression analysis.

5. Clustering and Visualization

Leiden clustering (resolution = 0.5) and UMAP projection were performed using the scVI latent space and a nearest-neighbor graph.

6. Gene Category Definitions

Marker genes were grouped into four functional categories: neuronal, neurotransmitter-related, heart-specific, and housekeeping. The neuronal and neurotransmitter genes were derived from a zebrafish intracardiac nervous system study by Pedroni et al. (2024) [5]. Heart-specific and housekeeping markers were manually curated from established literature and gene expression references. All gene markers were initially selected from zebrafish-based studies and then applied consistently across all datasets.These were used for expression comparisons using bar plots, dot plots, and heatmaps.

7. Chimerism Analysis

Souporcell was used on the human sample to infer donor and recipient clusters using BAM and barcode files. Cluster identities were assigned via differential expression analysis.

8. Software and Execution Environment

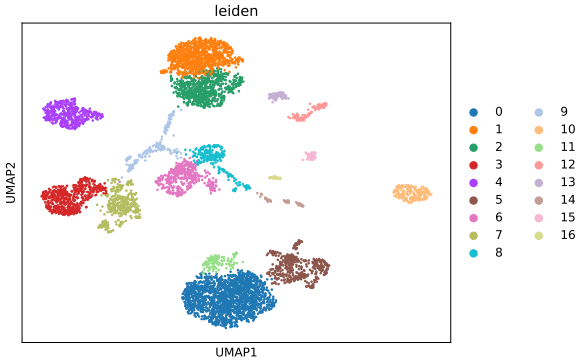
Python code was executed in Jupyter notebooks using Google Colab and VSCode. Cell Ranger and Souporcell were run on a Linux-based setup.

9. Reproducibility Statement

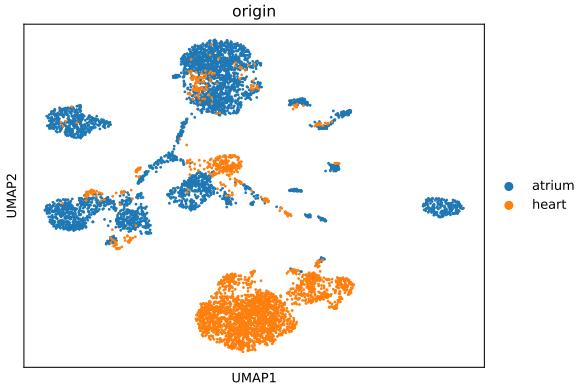
This project was based on publicly available datasets and carried out using well-documented, open-source tools. The methodology has been carefully structured to ensure that the analysis can be reproduced by following the detailed steps outlined in the report. The necessary steps for replicating the results are included in the methodology.

Results

Neuron-like gene expression in zebrafish

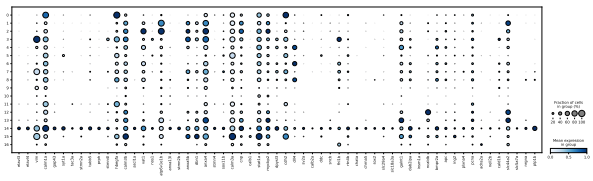


(a) Leiden clustering

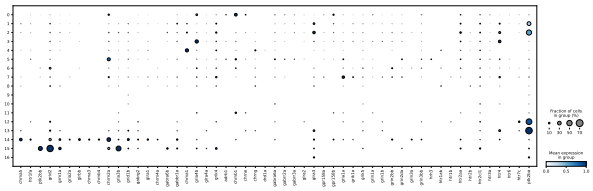


(b) Sample origin

Figure 1. UMAP projections of merged zebrafish heart samples.

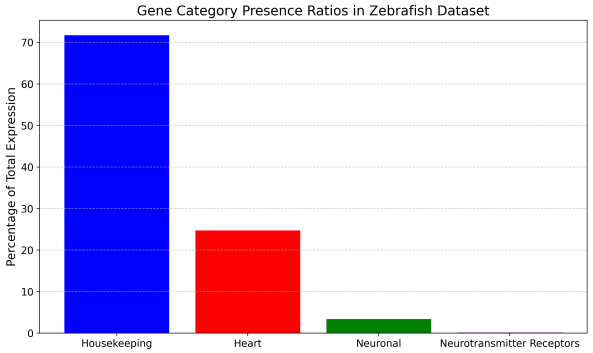


(a) Neuronal gene expression

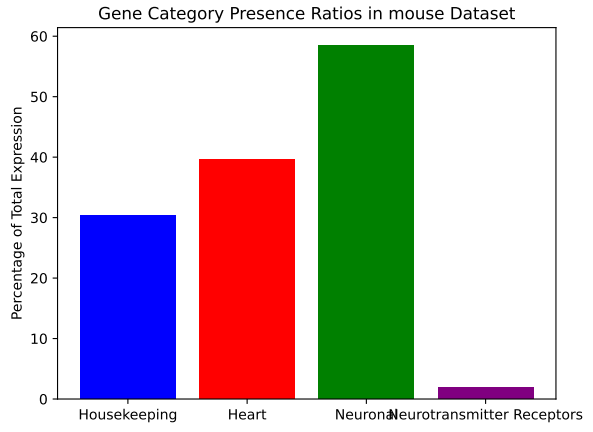


(b) Neurotransmitter gene expression

Figure 2. Dot plots of gene expression for neuronal and neurotransmitter genes in zebrafish.



(a) Zebrafish



(b) Mouse

Figure 3. Relative expression of gene categories across zebrafish and mouse datasets.

Several zebrafish heart clusters expressed neuronal genes including *elavl3*, *elavl4*, *calm1a*, *stat1a*, *cdh2*, *slc44a2*, *calm3b*, and *mycbp2*. Neurotransmitter-related genes such as *grid2*, *ptk2ba*, *gria3b*, *chrm2a*, *ptk2bb*, and *gria4b* were also detected, though with generally lower expression. These findings support the presence of neuron-like transcriptional features in zebrafish cardiac tissue. UMAP projections showed both the separation of Leiden clusters and the integration of atrium and whole-heart cells, supporting the dataset's combined analysis(Figure 1).

Neuron-like gene expression in mouse

In the mouse heart nuclei dataset, several clusters expressed neuronal genes including *Mycbp2*, *Plxna4*, *Galnt1*, *Apc*, and *Vim*. Neurotransmitter-related genes such as *Htr2b*, *Chrnb1*, and *Grik5* were also observed. While expression levels varied across clusters, these results point to the presence of neuron-like transcriptional

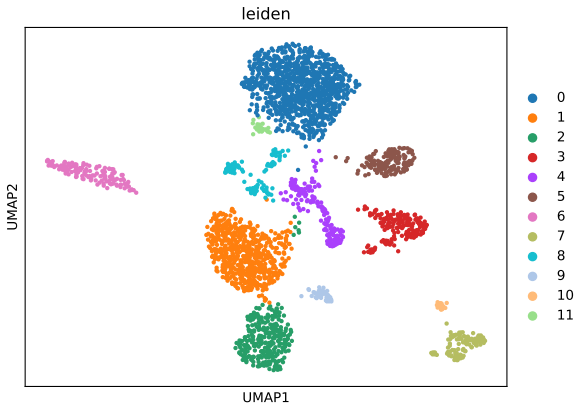
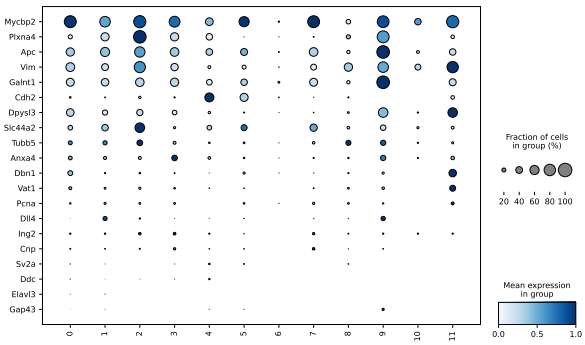
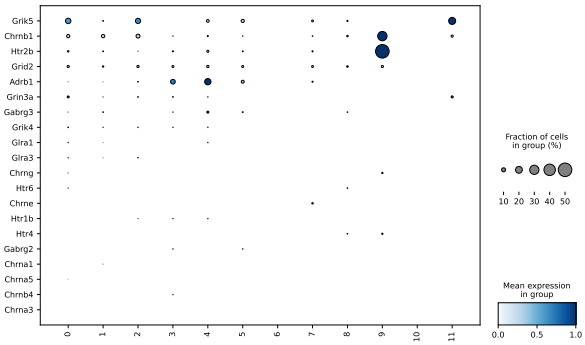


Figure 4. UMAP projection of mouse heart nuclei dataset.

signatures within specific cardiac cell populations. As shown in the gene category bar chart (Figure 3b), neuronal markers were more strongly expressed in mouse than in zebrafish.



(a) Neuronal gene expression

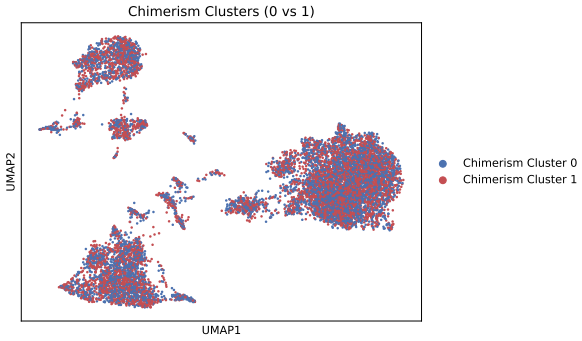


(b) Neurotransmitter gene expression

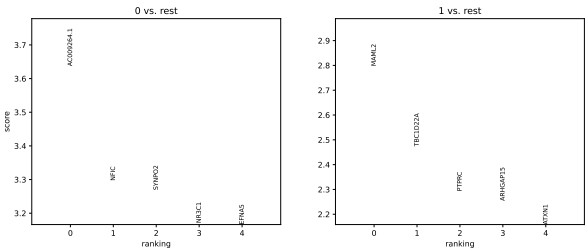
Figure 5. Dot plots of gene expression for neuronal and neurotransmitter genes in the mouse heart dataset.

Chimerism in Human Heart Transplant Tissue

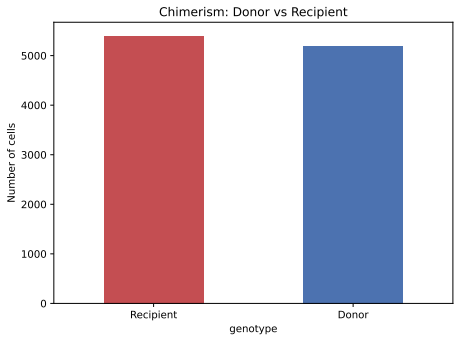
Souporcell analysis of the human heart transplant sample revealed two major cell populations, likely corresponding to donor and recipient origins. The UMAP visualization showed distinct but balanced clustering of these groups (Figure 6a), suggesting that both donor- and recipient-derived cells continue to coexist in the transplanted tissue. Differential expression analysis showed that one cluster expressed genes such as *PTPRC*, *TBC1D22A*, *MAML2*, *ARHGAP15*, and *ATXN1*, while the other showed higher expression of *NFIC*, *SYNPO2*, *NR3C1*, and *EFNA5* (Figure 6b). *PTPRC* (CD45), a marker of T and B cells [6], was strongly expressed in one cluster, supporting its classification as recipient-derived.



(a) Genotype-based clustering



(b) Top differentially expressed genes between clusters



(c) Chimerism cluster identities

Figure 6. Chimerism analysis of human heart transplant tissue showing UMAP projections and key marker genes.

Discussion

Several genes associated with neural plasticity were expressed in the zebrafish heart dataset, including *calm1a*, *calm3a*, *calm3b*, *gria3b*, *gria4b*, *elavl3*, *elavl4*, *cdh2*, *dpysl3*, *chrm2a*, and *grid2*. These genes are linked to key processes involved in synaptic plasticity, including calcium signaling, synaptic function, structural remodeling, and RNA regulation, suggesting that some zebrafish heart

cells may possess molecular machinery typically associated with neural plasticity.

In the mouse dataset, plasticity-linked expression was more limited but still detectable. Genes like *mycbp2*, *cdh2*, and *dpysl3* were highly expressed, while others such as *elavl3*, *elavl4*, and *gap43* were present at lower levels. Neurotransmitter-related genes (*grik5*, *chrnb1*, *htr2b*) were also observed. The higher ratio of neuronal gene expression observed in the mouse compared to zebrafish may partly reflect differences in gene marker coverage or dataset preprocessing, rather than true biological abundance. These technical factors can affect how strongly neuron-like features appear in category-level analyses such as bar plots. Despite these differences, both species showed similar patterns of plasticity-associated gene expression, with the zebrafish dataset revealing a broader and stronger expression of core plasticity genes. This discrepancy could be due to species differences, but also differences in data handling: the mouse dataset was preprocessed, while the zebrafish data were analyzed from filtered data after running Cell Ranger.

In the human heart transplant sample, chimerism analysis confirmed the coexistence of donor- and recipient-derived cells. The roughly equal proportions of these cells, as shown in Figure 6c, may be due to cardiac allograft vasculopathy (CAV), a form of chronic rejection where the recipient's immune system infiltrates the transplanted heart. Although neural markers were not assessed in that dataset, the presence of plasticity-associated transcripts in animal models invites future investigation into whether similar signatures exist in transplanted human tissues.

These findings suggest that cardiac cell integration may influence more than just tissue repair. The molecular programs observed could potentially facilitate donor-recipient cell communication beyond structural repair, and might even mediate cross-talk between the transplanted heart and the recipient's nervous system.

Challenges and Limitations

- **Marker Ambiguity:** Some neuronal markers may also be expressed in non-neuronal cell types, making it difficult to definitively identify neuron-like cells.

- **Species Differences:** Transcriptomic depth, gene orthology, and physiological context differ across zebrafish, mouse, and human, limiting direct comparisons.

- **Human Biological Complexity:** Humans have more complex emotional and cognitive systems, making it challenging to directly translate findings from zebrafish and mouse models to human biology, particularly in areas related to memory and emotion.

Author contributions

Sumaya Abdulrahman (S.A.): Conceptualization, Software, Formal analysis, Visualization, Writing – original draft.

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