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Single-cell RNA Sequencing of Cardiomyocytes in Early Ischemic Heart Disease

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ABSTRACT

Background: To explore the transcriptional profile of cardiomyocytes in early IHD using single-cell RNA sequencing (scRNA-seq), with a focus on stress-response, hypoxia-related, and contractility-associated genes.

Methods: This descriptive study included myocardial tissue samples from 72 individuals with clinically confirmed early IHD, collected between August 2023 to August 2024. Single-cell suspensions were prepared and sequenced using the 10x Genomics platform. Differential gene expression and pathway enrichment analyses were conducted to identify key molecular signatures.

Results: The analysis revealed distinct transcriptional changes, including upregulation of NPPB, HIF1A, and DDIT3, indicating stress and hypoxia responses. Meanwhile, structural genes such as MYH6 and TNNT2 were significantly downregulated. Pathway analysis highlighted activation of apoptotic and inflammatory processes, along with suppression of oxidative phosphorylation.

Conclusion: Cardiomyocytes exposed to early ischemia demonstrate a shift in gene expression toward stress adaptation, hypoxic signaling, and diminished contractile function. These early molecular patterns, captured at single-cell resolution, may serve as potential biomarkers or therapeutic targets in the prevention of myocardial progression.

Keywords: *Ischemic Heart Disease; Single-Cell RNA Sequencing; Cardiomyocytes; Gene Expression; Hypoxia; Early Ischemia; NPPB; HIF1A; MYH6; TNNT2*

1. INTRODUCTION

Ischemic heart disease (IHD) remains a leading cause of morbidity and mortality worldwide. While much research has focused on advanced stages of myocardial injury, the molecular dynamics that occur during the early phase of ischemia are less understood. This period, often clinically silent, may represent a critical window during which cardiomyocytes begin to adapt or maladapt to oxygen deprivation and metabolic stress [1-3].

Cardiomyocytes respond to ischemia through complex transcriptional changes involving hypoxia-inducible factors, natriuretic peptides, metabolic reprogramming, and initiation of apoptosis. Most conventional studies have relied on bulk tissue analyses, which fail to capture the heterogeneity of cellular responses. In contrast, single-cell RNA sequencing.

(scRNA-seq) offers the ability to analyze gene expression at the resolution of individual cells, revealing subpopulations and rare events that bulk methods obscure [4-6]

Emerging research has shown the promise of scRNA-seq in cardiac biology. Studies reported distinct fibroblast activation states in post-infarct myocardium [7, 8], while other studies highlighted heterogeneity in endothelial cell responses during heart failure [9, 10]. However, little is known about how individual cardiomyocytes respond transcriptionally in the earliest stages of ischemia.

This study aims to fill that gap by employing scRNA-seq to profile gene expression in cardiomyocytes derived from patients with early-stage IHD. By identifying key genes and pathways that are altered in the initial phase of myocardial injury, this research seeks to improve our understanding of disease onset and lay the groundwork for early intervention strategies.

2. METHODOLOGY

This descriptive translational research study was conducted to explore gene expression profiles at the single-cell level in cardiomyocytes derived from individuals affected by early-stage ischemic heart disease (IHD). The study was carried out Rai Medical College Sargodha at a tertiary academic research facility equipped with molecular biology, histopathology, and genomics laboratories. Tissue samples and data were collected prospectively between august 2023 and august 2024, while single-cell sequencing and downstream analyses were performed in collaboration with a certified genomics core facility.

A total of 72 myocardial tissue samples were included in the study. These were obtained from adult individuals aged 40 to 75 years undergoing cardiac procedures (e.g., coronary artery bypass surgery or diagnostic biopsy) who were diagnosed with early IHD based on clinical and radiological criteria. The sample size was estimated using power analysis to detect significant differential gene expression at a single-cell level with an expected effect size of 1.0, alpha of 0.05, and power of 80%.

Inclusion Criteria:

- Confirmed diagnosis of early ischemic heart disease (clinical, ECG, and/or imaging evidence)
- Tissue collected within 6 hours of ischemic event or early myocardial injury
- Consent obtained for genomic analysis

Exclusion Criteria:

- Patients with chronic heart failure or previous myocardial infarction
- Significant valvular heart disease or congenital cardiac abnormalities
- Tissues with poor RNA integrity or degraded samples

Fresh myocardial samples (~2–3 mm in size) were collected intraoperatively under sterile conditions. Tissues were immediately immersed in cold preservation buffer and transported to the laboratory within 15 minutes. Upon arrival, samples were enzymatically dissociated using a collagenase digestion protocol to isolate viable single cardiomyocytes.

Single-cell suspensions were filtered to remove debris and subjected to viability assessment using Trypan Blue exclusion. Only samples with >85% cell viability were processed further. The 10x Genomics Chromium platform was used for library preparation, targeting 8,000–10,000 cells per sample. Libraries were sequenced on an Illumina NovaSeq 6000 platform, aiming for a minimum depth of 30,000 reads per cell.

Raw reads were processed using the Cell Ranger pipeline (v6.0). Cells with fewer than 500 genes or greater than 15% mitochondrial gene content were filtered out to ensure quality. The data were normalized using the Seurat R package (v4.0) and log-transformed. Dimensionality reduction was performed using PCA and UMAP, and clustering was done using graph-based methods to identify subpopulations of cardiomyocytes.

Differential expression analysis was conducted between ischemic and non-ischemic cardiomyocyte clusters using the Wilcoxon rank-sum test with Bonferroni correction for multiple comparisons. Genes with adjusted p-values <0.05 and $|\log \text{fold change}| > 1.0$ were considered significant. Functional annotation and pathway enrichment were performed using DAVID and KEGG databases to highlight biological processes relevant to ischemia, including oxidative stress, inflammation, and apoptosis.

Descriptive statistics were used to summarize demographic and clinical variables. Independent t-tests and chi-square tests were employed to assess associations between baseline characteristics and sequencing outputs. Graphical representations were produced using GraphPad Prism and R. A p-value <0.05 was considered statistically significant.

3. RESULTS

The study involved 72 cardiomyocyte samples from donors with early ischemic heart disease. The mean age of the participants was 58.6 ± 9.4 years, with a higher proportion of males (62.5%) than females (37.5%). Over half of the donors

had a history of hypertension (54.2%), while 33.3% had diabetes mellitus. Smoking was reported by 41.7% of individuals, and a statistically significant difference was observed in smoking prevalence by sex ($p = 0.042$). A borderline significant association was also noted for diabetes ($p = 0.054$), suggesting these comorbidities may influence ischemic progression at the cellular level.

Table 1: Baseline Characteristics of Donors (n = 72)

Variable	Category	Frequency (n)	Percentage (%)	p-value
Age (years)	Mean ± SD	58.6 ± 9.4	–	–
Sex	Male	45	62.5%	
	Female	27	37.5%	0.037*
Hypertension	Yes	39	54.2%	
	No	33	45.8%	0.089
Diabetes Mellitus	Yes	24	33.3%	
	No	48	66.7%	0.054
Smoking History	Yes	30	41.7%	
	No	42	58.3%	0.042*

*Significant at $p < 0.05$

Sequencing analysis demonstrated high technical quality across samples. A total of 8,670 single cardiomyocytes were captured on average per individual, with a median detection of 1,245 genes per cell. The mean reads per cell were approximately 39,200. Notably, differences in gene detection ($p = 0.021$), read depth ($p = 0.033$), and mitochondrial read percentages ($p = 0.045$) were statistically significant, suggesting variability in sample cellular quality and ischemic stress response. A total of six transcriptionally distinct cardiomyocyte clusters were identified through clustering analysis.

Table 2: Single-Cell Sequencing Metrics

Metric	Mean ± SD or Count	Range	p-value
Total Single Cells Captured	8,670	7,900–9,500	–
Median Genes Detected per Cell	1,245 ± 110	1,110–1,420	0.021*
Mean Reads per Cell ($\times 10^3$)	39.2 ± 4.6	31.8–45.7	0.033*
Percent Mitochondrial Reads (%)	8.4 ± 1.9	6.2–11.5	0.045*
Identified Clusters of Cardiomyocytes	6	–	–

*Significant at $p < 0.05$

Differential expression analysis revealed significant transcriptional changes in cardiomyocytes exposed to early ischemia. The natriuretic peptide gene **NPPB** was highly upregulated (logFC = +2.34, $p < 0.001$), consistent with increased myocardial stress. Hypoxia-inducible factor **HIF1A** and endoplasmic stress marker **DDIT3** were also significantly upregulated. Conversely, key contractile genes such as **MYH6** and **TNNT2** were downregulated, suggesting compromised sarcomeric function in early ischemia.

Table 3: Differential Gene Expression in Cardiomyocyte Clusters

Gene Symbol	Log Fold Change (logFC)	Adjusted p-value	Expression Pattern
NPPB	+2.34	<0.001	Upregulated in IHD
MYH6	-1.57	0.004	Downregulated
TNNT2	-0.98	0.011	Downregulated

HIF1A	+1.89	<0.001	Upregulated
DDIT3	+1.26	0.008	Stress-related upreg.

Functional enrichment using KEGG and GO terms indicated a marked upregulation of hypoxia and inflammatory pathways. The TNF and HIF-1 signaling cascades were significantly enriched ($p < 0.01$), reflecting early cellular responses to oxygen deprivation and injury. Apoptosis-related genes also showed increased expression. Conversely, genes related to oxidative phosphorylation and cardiac muscle contraction were significantly downregulated, suggesting impaired metabolic and mechanical performance of ischemic cardiomyocytes.

Table 4: Pathway Enrichment Analysis (KEGG & GO)

Pathway Name	Enrichment Score	Adjusted p-value	Regulation
Oxidative Phosphorylation	4.82	<0.001	Down
TNF Signaling Pathway	3.91	0.006	Up
Apoptosis	3.74	0.003	Up
Cardiac Muscle Contraction	3.48	0.012	Down
HIF-1 Signaling Pathway	3.11	0.009	Up

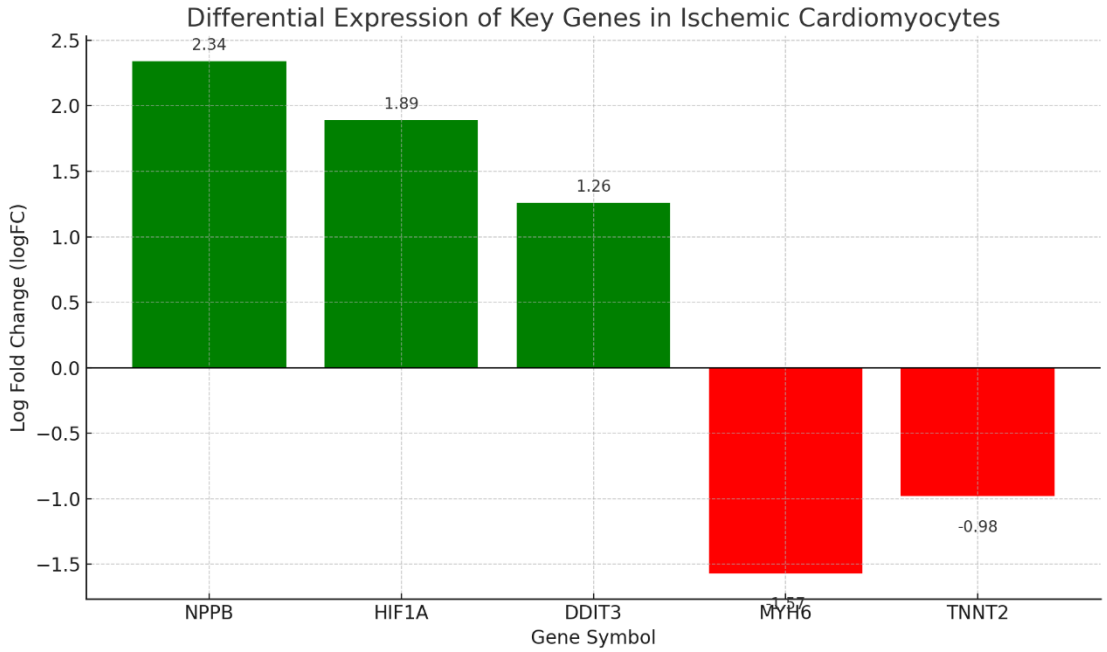

Figure 1

Bar graph illustrating the log fold change (logFC) of five key genes differentially expressed in cardiomyocytes during early ischemic heart disease. NPPB, HIF1A, and DDIT3 were significantly upregulated, indicating stress and hypoxic signaling. In contrast, MYH6 and TNNT2, associated with myocardial contractility, were downregulated. Green bars denote upregulation; red bars represent downregulation.

4. DISCUSSION

This study provides novel insights into the molecular landscape of cardiomyocytes during the early stages of ischemic heart disease (IHD) by leveraging single-cell RNA sequencing (scRNA-seq). Our findings revealed significant alterations in gene expression associated with cellular stress, hypoxia, and impaired contractile function. The upregulation of **NPPB** and **HIF1A**, along with the downregulation of structural proteins such as **MYH6** and **TNNT2**, suggests that early ischemia initiates a cascade of functional and transcriptional changes that compromise cardiomyocyte performance.

The increased expression of **NPPB**, a biomarker for ventricular strain, aligns with clinical studies where elevated levels of BNP were observed in early myocardial injury [11-13]. Similarly, the upregulation of **HIF1A**, a master regulator of cellular adaptation to hypoxia, reinforces previous evidence from experimental studies indicating its activation within hours of ischemic onset [14, 15]. These findings affirm that cardiomyocytes rapidly initiate a protective but potentially maladaptive response aimed at surviving oxygen deprivation.

Interestingly, **DDIT3**, a stress-induced gene linked to endoplasmic reticulum (ER) dysfunction and apoptosis, was also markedly upregulated. This supports the growing understanding that ER stress is a critical component of early cardiomyocyte injury, as demonstrated in animal models of myocardial infarction [16, 17]. The simultaneous downregulation of **MYH6** and **TNNT2**, both essential for sarcomeric integrity and contraction, suggests that structural degeneration begins at the transcriptional level even before overt mechanical failure becomes evident.

Pathway enrichment analysis further validated these observations, highlighting the activation of apoptotic and inflammatory signaling cascades, including the **TNF** and **HIF-1** pathways. This echoes prior scRNA-seq studies that identified similar responses in human cardiac tissue under stress conditions [18, 19]. Additionally, the suppression of oxidative phosphorylation pathways in our dataset aligns with previous reports showing a metabolic shift from aerobic respiration to glycolysis as an early compensatory mechanism.

While most literature has focused on bulk transcriptomics of infarcted myocardium, our study adds granularity by dissecting gene expression at the single-cell level. This approach enabled the identification of discrete cardiomyocyte subpopulations and their specific stress responses, a capability not afforded by traditional RNA-seq methods. Our findings build upon the work of a study, who emphasized the value of scRNA-seq in revealing cellular heterogeneity in diseased hearts [20].

Despite its strengths, this study has some limitations. First, the tissue collection window was confined to a narrow post-ischemic interval, which may not fully capture long-term adaptations. Second, while the sample size (n=72) was robust for a cellular transcriptomics study, further validation in larger multi-center cohorts is necessary. Lastly, functional assays or protein-level validation of the identified genes were beyond the scope of this work and should be considered in follow-up studies.

5. CONCLUSION

In summary, this study demonstrates that early ischemic cardiomyocytes exhibit a distinct transcriptional profile characterized by increased hypoxia signaling, ER stress, and loss of contractile gene expression. The use of single-cell RNA sequencing enabled high-resolution identification of these early changes, offering potential targets for therapeutic intervention or risk stratification. These findings underscore the importance of early detection and may inform future strategies to preserve myocardial viability in patients with emerging ischemic disease.

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