CHAMBER-SPECIFIC TRANSCRIPTOMIC SIGNATURES IN HUMAN HEART FAILURE WITH REDUCED EJECTION FRACTION: A COMPARATIVE RNA-SEQ ANALYSIS

Špecifické transkriptómové znaky v komorách srdca u pacientov s kardiálnym zlyhávaním so zníženou ejekčnou frakciou: komparatívna analýza RNA-seq

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Abstract
Background: Heart failure with reduced ejection fraction (HFrEF) is characterised by extensive molecular remodelling of the myocardium. However, the extent to which transcriptomic changes differ between the left and right ventricles remains incompletely defined.
Methods: We performed a comparative transcriptomic analysis of left and right ventricular myocardium from patients with HFrEF and non-failing (NF) donor controls using publicly available RNA sequencing data (GSE161472). Differential gene expression was analysed using a generalised linear model incorporating ventricular chamber, disease status and their interaction, with multiple testing correction applied.
Results: A total of 39,376 genes were analysed across 42 myocardial samples. Comparison between HFrEF and NF myocardium identified 2,199 differentially expressed genes (FDR < 0.05), of which 404 showed biologically meaningful expression changes ($|\log_2$ fold change$| > 1$). Most heart failure–associated transcriptional changes were shared between ventricles. Only one gene, HSPB1, demonstrated significant chamber-dependent regulation, with higher expression in the left ventricle in HFrEF.
Conclusions: Transcriptomic changes in HFrEF appear largely shared between left and right ventricular myocardium, with limited evidence of chamber-specific modulation in our cohort. These findings suggest that common molecular programmes predominate in advanced disease, while chamber-dependent differences may contribute more subtly to myocardial remodelling *(Fig. 2, Ref. 12). Text in PDF www.lekarskyobzor.sk.*
KEY WORDS: heart failure, reduced ejection fraction, left ventricle, right ventricle, transcriptome, RNA sequencing.
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Abstrakt
Pozadie: Srdcové zlyhávanie so zníženou ejekčnou frakciou (HFrEF) je charakterizované rozsiahlymi molekulárnymi prestavbami myokardu. Rozsah, v akom sa transkriptomické zmeny líšia medzi ľavou a pravou komorou, však zostáva nedostatočne objasnený.
Metódy: Vykonali sme komparatívnu transkriptomickú analýzu myokardu ľavej a pravej komory u pacientov s HFrEF a u nepostihnutých darcov (NF) s využitím verejne dostupných údajov RNA sekvenovania (GSE161472). Diferenciálna génová expresia bola analyzovaná pomocou generalizovaného lineárneho modelu zahŕňajúceho komoru, stav ochorenia a ich vzájomnú interakciu, pričom bola aplikovaná korekcia na viacnásobné porovnávanie.
Výsledky: Celkovo bolo analyzovaných 39 376 génov naprieč 42 vzorkami myokardu. Porovnanie medzi HFrEF a NF myokardom identifikovalo 2 199 diferencovane exprimovaných génov (FDR < 0,05), z ktorých 404 vykazovalo biologicky významné zmeny expresie ($|\log_2$ fold change$| > 1$). Väčšina transkripčných zmien asociovaných so srdcovým zlyhávaním bola spoločná pre obe komory. Iba jeden gén, HSPB1, vykazoval štatisticky významnú komorovo špecifickú reguláciu, s vyššou expresiou v ľavej komore u pacientov s HFrEF.
Záver: Transkriptomické zmeny pri HFrEF sa javia ako prevažne spoločné pre ľavú aj pravú komoru, s obmedzeným dôkazom komorovo špecifickej modulácie v našej kohorte. Tieto zistenia naznačujú, že v pokročilých štádiách ochorenia dominujú spoločné molekulárne programy, zatiaľ čo komorovo podmienené rozdiely môžu prispievať k remodelácii myokardu len miernejším spôsobom *(obr. 2, lit. 12). Text v PDF www.lekarskyobzor.sk.*
KĽÚČOVÉ SLOVÁ: srdcové zlyhávanie, znížená ejekčná frakcia, ľavá komora, pravá komora, transkriptóm, sekvenovanie RNA.
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Introduction

Heart failure (HF) is a major global health burden, affecting millions of individuals and contributing substantially to morbidity and mortality worldwide. It is a complex clinical syndrome characterised by impaired cardiac function and maladaptive remodeling of the my-

ocardium, often leading to progressive functional decline and reduced quality of life. Transcriptomic profiling of human heart tissue has provided important insights into the molecular mechanisms underlying HF and has identified coordinated gene expression changes associated with disease progression and adverse remodeling (1, 2).

The left and right ventricles of the heart differ substantially in embryologic origin, structural organisation, hemodynamic loading conditions, and metabolic demands. The left ventricle (LV) generates high systemic pressures, whereas the right ventricle (RV) operates within a low-pressure pulmonary circulation, resulting in distinct physiological and adaptive responses to stress. Accordingly, accumulating evidence indicates that LV and RV myocardium exhibit differences in cellular composition, metabolic regulation, and transcriptional programs, both under physiological conditions and in cardiovascular disease (1).

Despite these known distinctions, the extent to which HF–associated transcriptional remodelling differs between the left and right ventricles remains incompletely defined. Many transcriptomic studies have focused on pooled ventricular tissue or have primarily examined global disease-associated expression patterns, limiting the ability to assess chamber-specific effects (3, 4). More recent investigations using bulk and single-cell transcriptomic approaches suggest that HF may be characterised by a combination of shared molecular programmes and chamber-associated variation; however, the relative contribution of these components appears to vary across cohorts, disease stages and analytical strategies. As a result, the degree to which ventricular identity modulates gene expression in established HF remains uncertain (4).

In this context, we performed a comparative transcriptomic analysis of left and right ventricular myocardium obtained from patients with HFrEF and NF donor controls. By jointly modelling ventricular identity and disease status, we sought to examine whether disease-associated transcriptional changes are predominantly shared across chambers or whether chamber-dependent modulation can be detected within this framework. Through this approach, we aimed to provide a balanced assessment of ventricular-specific molecular patterns in human heart failure, while acknowledging the inherent constraints of bulk transcriptomic analysis.

Methods

Publicly available human myocardial transcriptomic data were obtained from the Gene Expression Omnibus (GEO) under accession number GSE161472. This dataset comprises bulk RNA sequencing profiles generated from LV and RV myocardial tissue collected from individuals with HFrEF and from NF donor controls. All data were de-identified and publicly accessible; therefore, institutional ethics approval was not required.

Raw gene-level count matrices were imported into R (version 4.5.2) and processed using the DESeq2 package (version 1.50.2). Sample-level metadata were curated from GEO records and used to annotate each sample according to ventricular origin (LV or RV) and disease status (HFrEF or NF). Only samples with complete annotation were retained for analysis. Following quality control, the final dataset comprised 42 myocardial samples (LV-NF, n = 9; RV-NF, n = 10; LV-HFrEF, n = 12; RV-HFrEF, n = 11).

Gene-level counts were normalised using the median-of-ratios method implemented in DESeq2 to account for sequencing depth and compositional differences. Differential expression analysis was performed using a negative binomial generalised linear model, which allows robust modeling of over dispersed count data typical of RNA sequencing experiments. To examine both global disease effects and chamber-specific responses, the statistical model incorporated ventricular chamber (LV vs RV), disease condition (HFrEF vs NF), and their interaction. This modeling framework enables simultaneous estimation of: (i) baseline transcriptional differences between the left and right ventricles, (ii) disease-associated changes within each ventricle, and (iii) chamber-specific modulation of gene expression in response to HF.

Statistical inference was performed using Wald tests, and multiple hypothesis testing was controlled using the Benjamini–Hochberg false discovery rate (FDR). Genes with an adjusted p-value < 0.05 were considered statistically significant. For descriptive purposes, effect size thresholds (absolute $\log_2$ fold change > 1) were additionally used to identify genes with biologically meaningful regulation. Gene identifiers were retained as Entrez Gene IDs, and annotation to gene symbols was performed using current NCBI gene annotations.

To visualise transcriptional differences, volcano plots were generated depicting $\log_2$ fold changes against $-\log_{10}$ adjusted p-values. These plots were used to illustrate both global disease-associated expression patterns and chamber-specific modulation of gene expression.

Results

Following quality control and filtering, a total of 39,376 genes were retained for downstream analysis. Differential gene expression analysis comparing HFrEF and NF myocardium identified 2,199 significantly regulated genes (FDR < 0.05). Among these, 404 genes demonstrated biologically meaningful changes, defined by an absolute $\log_2$ fold change greater than 1. Of these, 253 genes were upregulated and 151 were downregulated in HFrEF compared with NF myocardium.

The global pattern of transcriptional changes associated with HF is illustrated in the corresponding volcano plot (Figure 1). The distribution of effect sizes demonstrates a broad range of gene expression alterations, with both increased and decreased transcript abundance contributing to the overall remodeling profile observed in failing human myocardium.

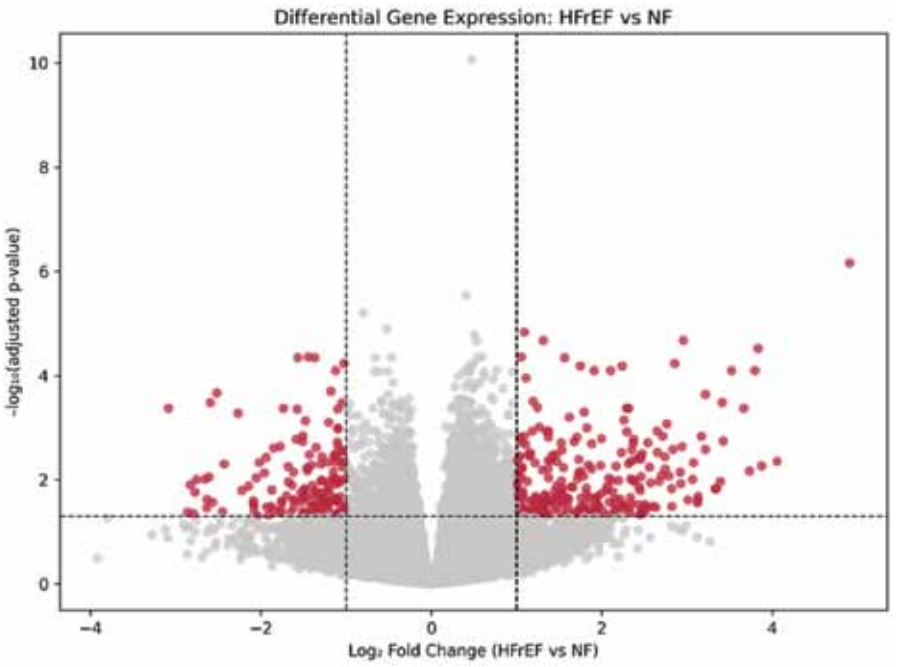

Figure 1. Differential gene expression between heart failure with reduced ejection fraction and non-failing myocardium (HFrEF: Heart failure with reduced ejection fraction, NF: Non-failing hearts).

To assess whether HF–associated transcriptional changes differed between ventricular chambers, we applied a statistical interaction model incorporating both disease status and ventricular identity. This approach enabled identification of genes exhibiting chamber-dependent modulation in the context of HF. The analysis revealed that the majority of transcriptional changes associated with HFrEF were shared between the left and right ventricles. Only a single gene, HSPB1, remained significant after correction for multiple testing, indicating differential regulation between chambers (Figure 2).

Specifically, HSPB1 expression was increased in HFrEF relative to NF myocardium, with a greater magnitude of induction observed in the LV compared with the RV. No additional genes met the predefined significance threshold for chamber-specific regulation.

Discussion

This work examined whether disease-associated gene expression changes in human HFrEF appear similar or different between left and right ventricular myocardium when both chamber identity and disease status are modelled together. A broad heart-failure signal was observed at the transcriptome level, which is consistent with contemporary human cardiac single-nucleus and integrative transcriptomic studies showing that advanced HF is accompanied by large, coordinated shifts in gene expression and cell-state programmes across the myocardium rather than isolated single-gene effects (2, 5,

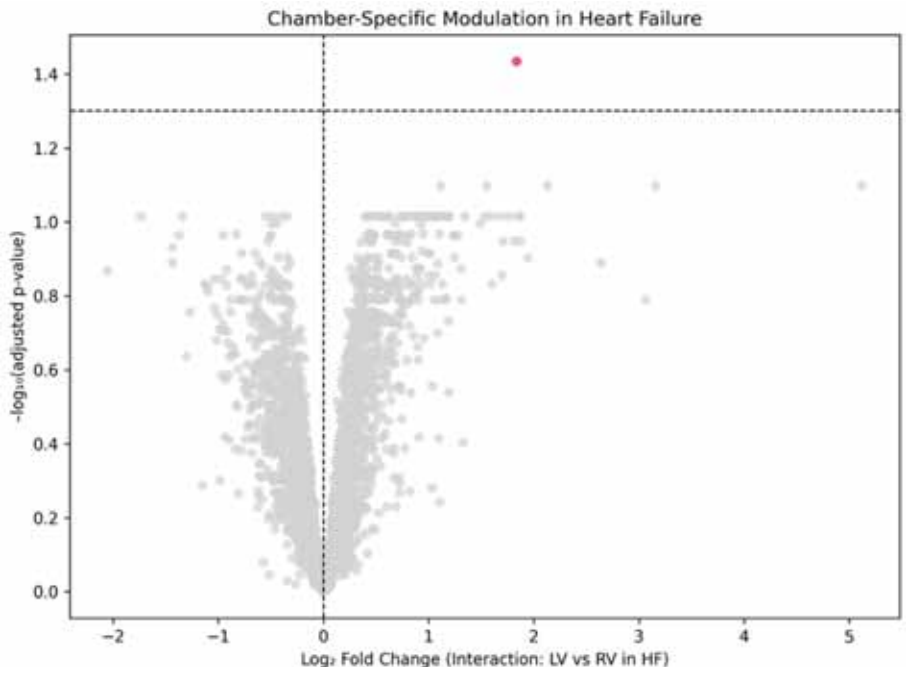

Figure 2. Chamber-dependent modulation of gene expression in heart failure (HF: Heart failure, LV: Left ventricle, RV: Right ventricle).

6). Against this background, the limited number of chamber-dependent findings in our interaction analysis should not be interpreted as evidence that the ventricles are transcriptionally indistinguishable; rather, it suggests that, within this dataset and modelling framework, the dominant component of expression change is shared across chambers, while chamber-modulated effects may be smaller in magnitude, more variable between individuals, or diluted in bulk tissue measurements. This interpretation is compatible with integrative efforts that pool large numbers of HF transcriptomes and emphasise consensus multicellular processes, particularly inflammation, fibrosis and remodelling, shared across HF cohorts and aetiologies (7).

Chamber identity differences are well described in the human heart under NF conditions, including regional and chamber-enriched transcriptional patterns reflecting developmental origin, haemodynamic load, and differences in cellular composition (8). However, in established HF, several high-resolution datasets suggest that disease drives both shared and cell-type-specific programmes, with cardiomyocytes often showing convergence toward disease-associated states while fibroblasts and immune populations diversify markedly; these dynamics can yield a strong global disease signature in bulk tissue while simultaneously increasing within-group heterogeneity that reduces power to detect interaction effects between chamber and disease (2, 5, 6). In practical terms, interaction testing is statistically demanding even when chamber-specific responses exist, they may not survive correction for multiple testing in modest cohorts because interaction terms have lower power than main-effect contrasts and are more sensitive to dispersion and sample heterogeneity. Contemporary spatial and single-cell studies also highlight that myocardial transcriptomes are strongly shaped by histological context and microanatomy, further supporting the view that bulk chamber-level comparisons can under-detect nuanced chamber-dependent biology when sample size is limited and tissue composition varies (9).

Within this framework, the emergence of HSPB1 as the single gene showing statistically significant chamber-dependent regulation after correction for multiple testing can be discussed as a plausible "high-signal" candidate rather than as an exclusive chamber marker. HSPB1 encodes heat shock protein beta-1 (Hsp27), a small heat shock protein involved in proteostasis and cytoskeletal stability and frequently linked to stress adaptation, oxidative injury responses and apoptotic regulation. Contemporary cardiovascular-focused reviews summarise *Hsp27/HSPB1* as a stress-responsive chaperone with multiple context-dependent roles in cardiovascular pathophysiology, including oxidative stress, inflammation and cell survival pathways (10). Experimental cardiomyocyte data also support mechanistic links between *HSPB1* and oxidative-stress handling: for example, work in cardiomyocyte-like cells has shown that oxidative stress can modify *HSPB1* and that *HSPB1*-related mechanisms may engage antioxidant signalling through *KEAP1/NRF2* pathways, providing a biologically coherent rationale for why *HSPB1* could track myocardial stress burden (11). In addition, broader human small heat shock protein biology underscores that functional consequences often depend on post-translational modification and oligomeric state, meaning that transcript-level differences should be interpreted as markers of programme activation rather than direct proof of functional protection or harm (12). Thus, a chamber-skewed increase of HSPB1 in HFrEF can be framed as potentially reflecting differential engagement of stress-response pathways between ventricles, plausibly related to differences in load and metabolic demand, without implying that HSPB1 alone "explains" chamber biology.

At the same time, it is important to keep the interpretation proportionate to what the data can support. Bulk RNA sequencing aggregates signals across cardiomyocytes, fibroblasts, endothelial cells, and immune populations; single-nucleus studies in human HF show substantial disease-associated shifts in cell composition (e.g., increased fibroblast-to-cardiomyocyte ratio) and marked cell-type-specific programmes that may differ by region and aetiology, potentially obscuring subtle chamber-by-disease interactions in bulk tissue analyses (2, 6). In addition, small cohort size, clinical heterogeneity, and technical factors in public datasets can increase dispersion and reduce reproducibility of weaker chamber-dependent effects after multiple-testing correction. Finally, for stress proteins such as *HSPB1*, transcript abundance may not track protein abundance or activity because function is strongly shaped by phosphorylation and other post-translational modifications; therefore, the present findings naturally motivate targeted follow-up at the protein level (including phosphorylation state) or cell-type-resolved profiling, rather than allowing strong mechanistic claims on their own (10, 12).

Taken together, the results are most consistent with a model in which HF–associated transcriptional remodelling in bulk ventricular tissue is dominated by shared disease programmes, while chamber-specific modulation, is comparatively modest and more readily detectable with larger cohorts and/or higher-resolution approaches. Recent integrative resources that combine bulk and single-cell HF datasets support this perspective by identifying consensus multicellular processes and showing that many disease signatures generalise across studies, even as finer-grained heterogeneity emerges at the cell-type and spatial level (7, 9). Within the constraints of this dataset, HSPB1 stands out as a plausible chamber-modulated stress-response transcript, but its biological and clinical relevance will require validation in larger cohorts and with approaches that can separate cell-type effects and quantify protein-level regulation (10, 11).

Conclusion

Transcriptomic profiling of left and right ventricular myocardium in HFrEF in our cohort suggests that disease-associated gene expression changes are largely

shared between chambers. Only limited chamber-dependent modulation was observed, with HSPB1 emerging as the sole gene showing differential regulation, indicating that chamber-specific effects may be subtle relative to the broader transcriptional response associated with advanced disease. These findings are consistent with the notion that, at this stage of HF, common molecular programmes may predominate across ventricular compartments, while more nuanced chamber-specific patterns may remain difficult to detect using bulk transcriptomic approaches. Interpretation of these results should take into account the modest sample size, potential tissue heterogeneity and the influence of post-transcriptional regulatory mechanisms.*

*__Compliance with Ethics Requirements:__ Authors declare no conflict of interest regarding this article. The authors declare, that all the procedures and experiments of this research respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008 (5), as well as the national law.

Conflict of interest: The authors declare no conflict of interest.

Informed consent: Informed consent was obtained from all individual participans included in the study.

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