RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

DOI: 10.4274/atfm.galenos.2024.20092
Ankara Üniversitesi Tıp Fakültesi Mecmuası 2024;77(4):410-415

BASIC MEDICAL SCIENCES / TEMEL TIP BİLİMLERİ

TGF-β and Wnt Pathways Underlying the Pathophysiology of Degenerative Mitral Valve Regurgitation

Dejeneratif Mitral Yetmezliği Patofizyolojisinde Yer Alan TGF-β ve Wnt Sinyal Yolakları

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Abstract

Objectives: In aging society, hospitalizations and mitral valve surgeries are becoming more common due to progressive mitral valve diseases that ultimately lead to heart failure. In developing countries, degenerative mitral valve regurgitation (DMVR) is the leading cause of mitral regurgitation requiring surgical treatment. Nevertheless, a more profound understanding of the underlying pathological processes via a molecular biology approach could improve clinical management. In this study, bioinformatic analyses were performed to elucidate the underlying pathophysiological molecular mechanisms using transcriptome data of atrial tissues from patients with DMVR.

Materials and Methods: Bioinformatic analysis were done using GSE115574 dataset from The Gene Expression Omnibus (GEO) database. Transcriptome data which were downloaded from GEO database in CEL files generated from human left atrium and right atrium tissues in patients with DMVR in sinus rhythm (n=16) was used to perform bioinformatic analysis. Gene expression microarray analysis have done on Affymetrix platform previously. Bioinformatics, gene ontology, and functional enrichment analysis have been conducted on Partek GS V7.0 and WebGestalt software.

Results: The findings of this study reveal that multiple genes and various pathways play a role in the pathophysiology of DMVR. Among all transforming growth factor-beta (TGF-β) and Wnt signaling pathways enlightened according to false discovery rate thresholds and enriched geneset values. Prominent genes that involved in TGF-β and Wnt signaling pathways were *WNT5A*, *PLCB1*, *SFRP1*, *SMAD6*, *PITX2*, *SMAD7*, *ID2*, *BMP5*.

Conclusion: It's important to crystallize the molecular mechanisms of DMVR to facilitate early detection and develop targeted interventions. Management of TGF-β and Wnt signaling pathways in correct direction may offer solutions to slow DMVR progression and prevent it from becoming more complex.

Keywords: Mitral valve, TGF-β signaling pathway, Wnt signaling pathway

Öz

Amaç: Yaşlanan toplumlarda, en nihayetinde kalp yetmezliğine ilerleyen progresif mitral kapak hastalıkları nedeniyle hastaneye yatışlar ve mitral kapak ameliyatları daha yaygın hale gelmiştir. Gelişmekte olan ülkelerde, dejeneratif mitral kapak yetersizliği (DMVR) cerrahi tedavi gerektiren mitral yetersizliğinin önde gelen nedenidir. Bununla birlikte, altta yatan patolojik süreçlerin moleküler biyoloji yaklaşımıyla daha derinlemesine anlaşılması klinik yönetimi iyileştirebilir. Bu araştırmada, DMVR'li hastalardan alınan atriyum dokularının transkriptom verisi kullanılarak, altta yatan patofizyolojik moleküler mekanizmaları aydınlatmak amacıyla biyoinformatik analizler yapılmıştır.

Gereç ve Yöntem: Biyoinformatik analizler, Gen Ekspresyon Omnibus (GEO) veritabanından GSE115574 veriseti kullanılarak gerçekleştirilmiştir. Sinüs ritmindeki DMVR hastalarında (n=16) insan sol atriyum ve sağ atriyum elde edilen ve GEO veritabanından CEL dosyaları olarak indirilen transkriptom verisi biyoinformatik analizleri yapmak için kullanılmıştır. Gen ekspresyon mikroarray analizleri daha öncesinde Affymetrix platformunda gerçekleştirilmiştir. Biyoinformatik, gen ontolojisi ve fonksiyonel zenginleştirme analizleri Partek GS V7.0 ve WebGestalt yazılımlarında gerçekleştirilmiştir.

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Received/Geliş Tarihi: 27.05.2024 **Accepted/Kabul Tarihi:** 26.12.2024

Bulgular: Bu çalışmanın bulguları, DMVR patofizyolojisinde birden fazla genin ve çeşitli yolakların rol oynadığını ortaya koymaktadır. Tüm dönüştürücü büyüme faktörü-beta (TGF-β) ve Wnt sinyal yolakları arasında yanlış keşif oranı eşiklerine ve zenginleştirilmiş gen seti değerlerine göre aydınlatılmıştır. TGF-β ve Wnt sinyal yolaklarında yer alan öne çıkan genler *WNT5A*, *PLCB1*, *SFRP1*, *SMAD6*, *PITX2*, *SMAD7*, *ID2*, *BMP5* olarak tespit edilmiştir.

Sonuç: Erken teşhisi kolaylaştırmak ve hedefe yönelik müdahaleler geliştirmek için DMVR'nin moleküler mekanizmalarının tam olarak aydınlatılması önemlidir. TGF-β ve Wnt sinyal yolaklarının doğru şekilde yönetilmesi, DMVR progresyonunu yavaşlatmak ve daha karmaşık hale gelmesini önlemek için çözümler sunabilir.

Anahtar Kelimeler: Mitral kapak, TGF-β sinyal yolağı, Wnt sinyal yolağı

Introduction

Degenerative mitral valve regurgitation (DMVR) occurs leaflet malcoaptation during systole, leads to abnormal regurgitant flow from left ventricle to left atrium (LA). DMVR is a common valvular disorder characterized by the dysfunction of the mitral valve, resulting in the backward flow of blood from the left ventricle to the LA during systole. Hospitalizations and mitral valve surgeries have risen because of DMVR which is a progressive disease that eventually develops heart failure in the aging society (1). In developing countries, DMVR is the primary reason for mitral regurgitation that necessitates surgical intervention (2). Most common mechanisms underlying pathophysiology of DMVR are either fibroelastic deficiency of mitral valve or myxomatous degeneration of the mitral leaflets as in Barlow's disease (2). Remarkable consequences of DMVR are LA pressure and volume overload that leads to LA enlargement, a possible precursor of atrial fibrillation (AFib) due to the structural and electroanatomical remodeling. AFib is the most common arrhythmia and prevalence of AFib increases with aging (3). Also AFib has a negative progressive influence on DMVR prognosis (4). Chronic DMVR causes progressive LA enlargement, which negatively affects cellular coupling and electrical conduction and onsets AFib (5).

Current guidelines for intervention are mainly based on hemodynamic factors. However, a deeper insight into the underlying pathological processes through a molecular biology approach could enhance clinical management (6). In this study, we analysed transcriptomes of atrium tissues of DMVR patients using bioinformatic analyses. Differentially expressed genes were enriched in transforming growth factor-beta (TGF-β) and Wnt signaling pathways. The available knowledge identifies potential initial signaling pathways related to mechanical and biochemical triggers. These pathways determine the activation of specific transcription factors and subsequently modulate gene expression (7). DMVR is influenced by serotonin, TGF-β, and developmental pathways linked to heart valves, bones, and cartilage, such as bone morphogenetic protein (BMP) and Wnt signaling (8). Additionally, processes resembling osteogenesis and chondrogenesis may drive degenerative changes in aortic and mitral valves. Other implicated pathways include Notch, nitric oxide, and angiotensin II (9,10). The data of this study showed that gene expression profiles in atria of DMVR patients changed in different signaling pathways. Understanding the role of TGF-β and Wnt signaling pathways in the pathophysiology of degenerative mitral valve regurgitation is crucial for developing effective therapeutic strategies for this condition.

Materials and Methods

The raw microarray data, stored in CEL format files coming from transcriptomics of LA and RA tissues from 16 DMVR patients with sinus rhythm which were used as material that downloaded from Gene Expression Omnibus (GEO) database. Since CEL files downloaded from an open access functional genomics database were used as material in this study, there was no need for an ethics statement. The CEL files were uploaded to the Partek Genomic Suite® (PGS, V7.0, St. Louis, MO) (11).

Statistical Analysis and Bioinformatics

The preprocessing of the probe-level data was conducted and transformed using the Robust Multiarray Analysis algorithm. A fold change threshold of >1.5 and a p-value of <0.05 were set to identify differentially expressed transcripts. Adjusted p-values, known as q-values, were computed based on an optimized false discovery rate (FDR). A significance threshold for FDR was set at q<0.05. Furthermore, unsupervised hierarchical clustering was used to investigate the relationships between the LA and right atrial (RA) groups. Table listing the differentially expressed genes (DEGs) from LA versus RA comparison are available in Table 1.

Functional enrichment analysis were conducted using DEGs. Transcript IDs (Affy IDs) differentially regulated between LA and RA groups were uploaded to the WebGestalt (WEB-based Gene SeT AnaLysis Toolkit, http://www.webgestalt.org/) software. This platform was used for the annotation, enrichment, and visualization of these genes. gene ontology and pathway analyses were carried out using the WebGestalt toolkit. The functional enrichment analysis was executed using the overrepresentation analysis method and included adjustments for multiple tests with an FDR threshold of 0.05.

Results

The results of this study expose that several genes and different pathways are involved in the pathophysiology of DMVR. All patients participated to this study were in sinus rhythm during their operation (11). Atrium tissue transcriptomes were compared according to adjusted p-value <0.05 and fold change ±1.5 threshold. One hundred and ninety-eight Affy IDs were differentially expressed while 138 downregulated and 60 upregulated on Partek GS.

Functional enrichment analysis of DEGs from LA and RA tissues indicated that several KEGG pathways enlightened. Besides TGF-β and Wnt signaling pathways which are the focus of this study, retinol metabolism, glucagon signaling pathway, cell adhesion molecules, and chemokine signaling pathways are also enriched. But the geneset values or FDR thresholds were lower.

The genes from differentially expressed transcripts enriched in TGF-β and Wnt signaling pathways can be seen in Table 1. Affinity propagation of terms analysed by overrepresentation analysis are shown as volcano plot in Figure 1.

Table 1: The genes from differentially expressed transcripts enriched in TGF-β and Wnt signaling pathways

Affy ID	Gene symbol	Gene name	Entrez gene	Pathway geneset enriched
205990_s_at	*WNT5A*	Wnt family member 5A	7474	Wnt signaling pathway
213425_at	*WNT5A*	Wnt family member 5A	7474	Wnt signaling pathway
213222_at	*PLCB1*	Phospholipase C-β 1	23236	Wnt signaling pathway
202037_s_at	*SRFP1*	Secreted Frizzled related protein 1	6422	Wnt signaling pathway
219908_at	*DKK2*	Dickkopf Wnt signaling pathway inhibitor 2	27123	Wnt signaling pathway
210220_at	*FZD2*	Frizzled class receptor 2	2535	Wnt signaling pathway
207069_s_at	*SMAD6*	SMAD family member 6	4091	TGF-β signaling pathway
207558_s_at	*PITX2*	Paired like homeodomain 2	5308	TGF-β signaling pathway
204790_at	*SMAD7*	SMAD family member 7	4092	TGF-β signaling pathway
201565_s_at	*ID2*	Inhibitor of DNA binding 2	3398	TGF-β signaling pathway
205431_s_at	*BMP5*	Bone morphogenetic protein 5	653	TGF-β signaling pathway

TGF-β: Transforming growth factor beta

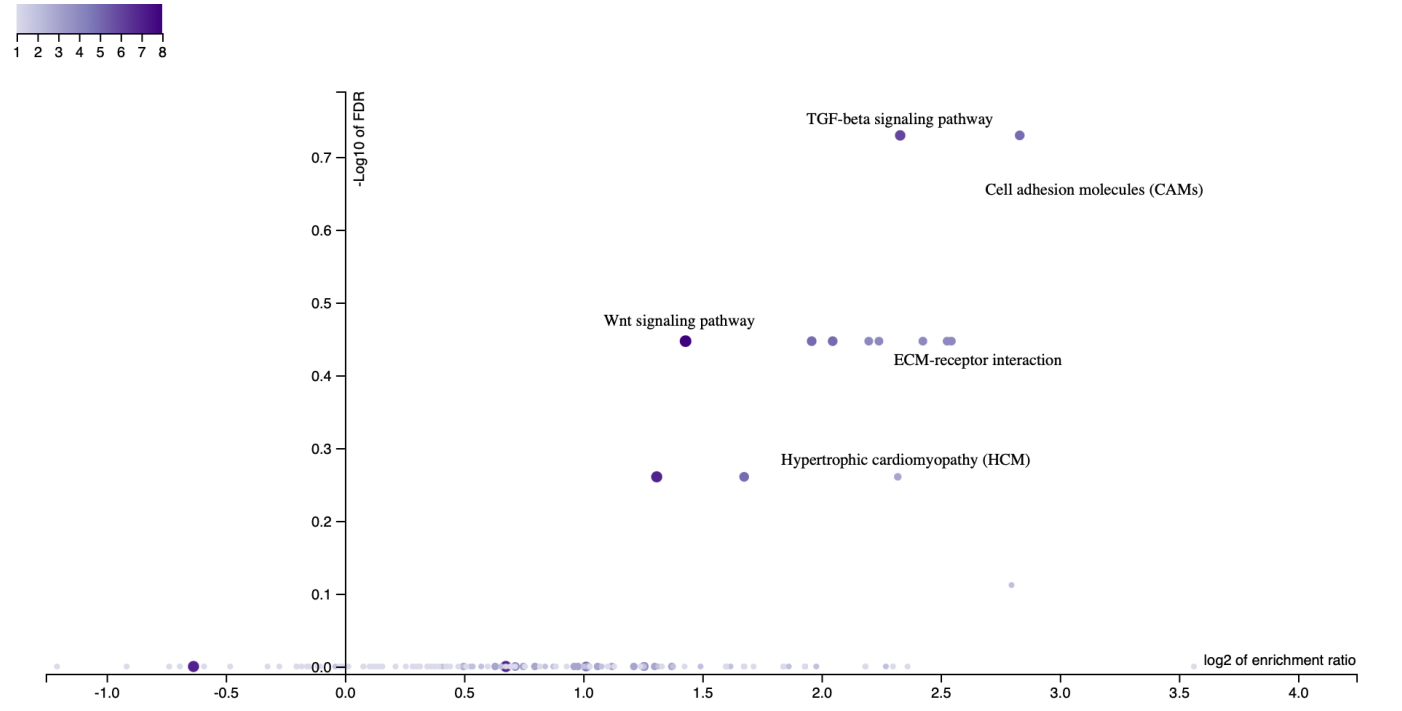

Figure 1: Affinity propagation of terms analysed by overrepresentation analysis are shown as volcano plot

ECM: Extracellular matrix, TGF: Transforming growth factor

Discussion

Reasons for progressing mitral regurgitation are divided into ischemic, which results from coronary artery disease, and nonischemic. The most frequent nonischemic causes of mitral regurgitation are degenerative in developed countries and, rheumatic in developing countries (12). During the early stages of valve degeneration, infiltrated inflammatory cells are frequently observed alongside elevated levels of proinflammatory cytokines like tumor necrosis factor-α, and interleukin-1β.

TGF-β Signaling Pathway

TGF-β is involved in various conditions including cardiac abnormalities, cardiac fibrosis, heart failure, chamber remodeling, and cardiac hypertrophy. The isoforms of TGF-β, along with activins, stimulate intracellular signaling through the SMAD2/3 transcription factors (13). Within the growth factor superfamily, the main subfamilies include TGF-β1, -β2, -β3 isoforms, BMPs, inhibins, and activins. Notably, the overexpression of TGF-β has been shown to play a significant role in various cardiac diseases characterized by fibrosis (14).

Differentially expressed geneset of this study's data functionally enriched in TGF-β signaling, which can be found on Kyoto Encyclopedia of Genes and Genomes pathway. TGF-β signaling is initiated by the dimerization of TGF-β, followed by its binding to TGF-β receptor type I (TGF-βR1) and type II (TGF-βR2). Specifically, numerous studies have identified elevated levels of TGF-β and increased expression of TGF-βR1 and TGF-βR2 in mitral regurgitation, observed in both human subjects and in vivo models (15,16). These findings have been thoroughly documented, establishing TGF-β as a key contributor to the myofibroblastic activation of valve interstitial cells when under tensile stress (17). This interaction forms a receptor heterocomplex that can recruit and phosphorylate SMAD proteins, thereby activating a variety of signal transduction pathways. SMAD6-7, molecules acting as inhibitors of SMAD1-5-8 play a significant role in preventing calcification (18). In this study, SMAD6 and SMAD7 are both downregulated which means TGF-β signaling inhibition is reduced in LA in DMVR patients. SMAD proteins are the major actor molecules in the TGF-β signaling pathway. Nuclear SMAD complexes work alongside DNA-binding transcription factors, as well as chromatin modifiers, to either positively or negatively influence the expression of genes responsive to TGF-β (19). The Wnt signaling pathway is a critical downstream pathway involved in TGF-β-mediated myocardial fibrosis. TGF-β triggers the secretion of Wnt and activates the Wnt/β-catenin signaling via the TGF-β activated kinase-1, which promotes the differentiation of myofibroblasts leading to myocardial fibrosis (20).

Wnt Signaling Pathway

There are two versions of Wnt signaling: canonical and non-canonical. Wnt signaling involves at least 19 Wnt ligands and 10 Frizzled receptors in humans, with the canonical pathway activated by Wnt ligands binding to Frizzled and either LRP5 or LRP6. This interaction stabilizes β-catenin, allowing it to accumulate and enter the nucleus to regulate gene expression through T cell-specific transcription factor/lymphoid enhancer-binding factor 1 (TCF/LEF) transcription factors. The Wnt signaling pathway consists of different components: the extracellular matrix, membrane, cytoplasmic part, and nuclear segments. Extracellular signals primarily involve *Wnt* genes like *Wnt3A*, *Wnt1*, and *Wnt5A*. The membrane component predominantly includes the Wnt receptors Frizzled (a specific seven-transmembrane receptor) and LRP5/6. The cytoplasmic component chiefly comprises β-catenin, the dishevelled genes (*DVL1*, *DVL2*, *DVL3*), glycogen synthase kinase-3β, and casein kinase I. The nuclear component mainly features β-catenin, which moves into the nucleus, members of the TCF/LEF family, and downstream target genes of β-catenin, such as MMPs and c-Myc. In canonical Wnt pathway dishvelled genes induces clustering of *LRP5/6*, Wnt, and Frizzled. Subsequently, balances β-catenin in the cytoplasm and *DVL* in the nucleus acts as a transcriptional regulator of *Wnt* target genes.

Wnt signaling plays a crucial role in embryogenesis and is activated when Wnt binds specifically to the Frizzled receptor and is stabilized by low-density lipoprotein receptor-related protein LRP5/6. This activation of the canonical Wnt pathway results in decreased degradation of β-catenin, which then moves to the nucleus to activate the TCF/LEF (21). Mutations in Wnt5A lead to mild heart defects by disrupting cell-cell adhesion and the organization of the cytoskeleton in cardiomyocytes as they differentiate (22). Wnt5A is a key player of non-canonical pathway and has been implicated to atherosclerotic progression (23).

Extracellular Wnt ligands can engage with various key antagonists that are secreted as well. Notably, these antagonists include the secreted Frizzled related proteins (SFRPs) and the Dickkopf proteins. The results of this study demonstrated that inhibitors of Wnt signaling were downregulated such as SFRP1 and DKK2.

Wnt signaling, which is essential for processes like bone and cartilage development, is regulated by at least five protein families that inhibit Wnt activity by targeting ligands, Frizzled, or LRP receptors (24). Specific ligands, such as Wnt3A and Wnt5A, are associated with chondrogenesis, while others promote osteogenesis (25). Studies showed that LRP5 mediates bone formation through inhibition of serotonin which directly

inhibits osteoblastogenesis (26). Dysregulated Wnt-LRP-5-β-catenin signaling has been implicated in calcific aortic valve disease, linking Wnt pathways to degenerative changes in valve tissues. Degenerative heart valve disease may mimic bone formation, resulting in osteogenesis in calcific aortic valves and chondrogenesis in myxomatous mitral valves (27). Recent findings suggest that serotonin, known to inhibit osteogenesis, might influence in this process by promoting chondrogenesis through serotonin expression in mitral valves which is leading to upregulation of TGF-β1, potentially preventing osteogenic changes (28). TGF-β1 upregulation enhances the production of extracellular matrix components like collagen and glycosaminoglycans.

Wnt signaling influences multiple pathways. In this study the expression of several modulators of the Wnt signaling pathway, such as Wnt5A, DKK2, and SFRP1, has been found differentially regulated in patients with DMVR.

The results of this study revealed increased regulation of TGF-β and Wnt signaling pathways in DMVR patient's atrial tissues. Understanding the triggers of degenerative valve disease and unraveling the complex interactions of signaling pathways may lead to the development of therapies targeted these pathways' triggers to slow or prevent disease progression.

Study Limitations

Bioinformatic enrichment tools are crucial for identifying, annotating, and analyzing large gene lists generated by high-throughput technologies like microarrays. While there may be minor variations between tools, the overall results are generally consistent, though subtle differences might occasionally be overlooked. Transcriptome profiling provides a precise snapshot of genome-wide mRNA expression at a precise moment and under the harvested time conditions but lacks predictive capability for downstream processes like translation, protein structures, interactions, and regulatory mechanisms. To address these limitations, complementary approaches such as protein interaction analysis are necessary to gain deeper insights. Additionally, global gene expression profiling using human or animal tissues faces challenges, particularly in accounting for metabolic processes influencing gene expression, a limitation inherent to tissue biopsy studies.

Conclusion

Degenerative calcific valve diseases are underestimated health issues. As the population ages and the absence of effective drug treatments, these conditions are expected to place an increasingly heavy strain on the global health system in the coming decades. Surgery option is just is to treat symptoms leaving the underlying pathophysiological mechanisms untouched. Further investigation should be done on clarifying molecular mechanisms underlying DMVR pathophysiology to detect disease in earlier stages and figure out intervention strategies on molecular triggers.

Ethics

Ethics Committee Approval: Since CEL files downloaded from an open access functional genomics database were used as material in this study, there was no need for an ethics statement.

Informed Consent: In this study, we analysed transcriptomes of atrium tissues of degenerative mitral valve regurgitation patients using bioinformatic analyses.

Acknowledgements

I would like to thank Prof. MD Ahmet Rüçhan Akar, Prof. MD Mustafa Serkan Durdu, Prof. MD Hilal Özdağ Sevgili, Prof. MD Yeşim Doğan for their support.

Footnotes

Financial Disclosure: This study received no financial support.

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