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ORIGINAL ARTICLE / ÖZGÜN MAKALE

NETWORK TOXICOLOGY FOR THE CARDIOVASCULAR TOXICITY ANALYSIS OF TYROSINE KINASE INHIBITORS

TİROZİN KİNAZ İNHİBİTÖRLERİNİN KARDİYOVASKÜLER TOKSİSİTE ANALİZİ İÇİN AĞ TOKSİKOLOJİSİ

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ABSTRACT

Objective: *This study aims to explore potential molecular mechanisms and targets of cardiovascular toxicities caused by tyrosine kinase inhibitors. Therefore, toxicogenomic data mining was conducted focusing on sunitinib, sorafenib, pazopanib, axitinib, and their associations with cardiovascular diseases.*
Material and Method: *Common genes between tyrosine kinase inhibitors and cardiovascular diseases were uncovered via comparative toxicogenomic databases. Additionally, protein-protein and gene-gene interactions were identified using STRING and GeneMANIA, respectively. Subsequently, hub proteins associated with tyrosine kinase inhibitor-induced cardiovascular diseases were determined through Metascape. Transcription factors and microRNAs related to this toxicity were identified using ChEA3 and MIENTURNET, respectively. Finally, gene ontology enrichment analysis and the most associated molecular pathways were identified using the DAVID database and Metascape, respectively.*
Result and Discussion: *Toxicogenomic data mining revealed six genes common between tyrosine kinase inhibitors and cardiovascular diseases, with five of these genes (FLT1, FLT4, KDR, MAPK1, and MAPK3) identified as hub genes. Physical interaction was dominant among these hub genes (77.64%). Sunitinib, sorafenib, pazopanib, and axitinib generally downregulated the activities of these proteins. SOX17 and SOX18 were prominent among transcription factors, while hsa-miR-199a-3p was the most important microRNA associated with this toxicity. Moreover, the Ras signaling pathway was mostly associated with tyrosine kinase inhibitor-induced cardiovascular toxicities. These findings make a substantial contribution to understanding the processes underlying cardiovascular diseases induced by sunitinib, sorafenib, pazopanib, and axitinib. They also reveal novel potential therapeutic targets, including genes, proteins, transcription factors, microRNAs, and pathways.*
Keywords: *Cardiovascular disease, in silico data mining, tyrosine kinase inhibitors*

ÖZ

Amaç: *Bu çalışma, tirozin kinaz inhibitörlerinin neden olduğu kardiyovasküler toksisitelerin potansiyel moleküler mekanizmalarını ve hedeflerini araştırmayı amaçlamaktadır. Bu nedenle, sunitinib, sorafenib, pazopanib, axitinib ve bunların kardiyovasküler hastalıklarla ilişkilerine odaklanarak toksikogenomik veri madenciliği yapılmıştır.*
Gereç ve Yöntem: *Tirozin kinaz inhibitörleri ile kardiyovasküler hastalıklar arasındaki ortak*

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genler, karşılaştırmalı toksikogenomik veritabanları aracılığıyla belirlenmiştir. Ayrıca, protein-protein etkileşimleri ve gen-gen etkileşimleri sırasıyla STRING ve GeneMANIA kullanılarak belirlenmiştir. Daha sonra, tirozin kinaz inhibitörü ile ilişkilendirilmiş kardiyovasküler hastalıklara ait merkezi proteinler Metascape kullanılarak belirlenmiştir. Bu toksisite ile ilişkili transkripsiyon faktörleri ve mikroRNA'lar sırasıyla ChEA3 ve MIENTURNET kullanılarak belirlenmiştir. Son olarak, gen ontolojisi zenginleştirme analizi ve en çok ilişkilendirilen moleküler yollar sırasıyla DAVID veritabanı ve Metascape kullanılarak belirlenmiştir.

Sonuç ve Tartışma: *Toksikogenomik veri madenciliği, tirozin kinaz inhibitörleri ile kardiyovasküler hastalıklar arasında altı ortak geni ortaya çıkardı; bunlardan beşi (FLT1, FLT4, KDR, MAPK1 ve MAPK3) merkezi genler olarak belirlendi. Bu merkezi genler arasında fiziksel etkileşim baskın olarak gözlemlendi (%77.64). Sunitinib, sorafenib, pazopanib ve axitinib genel olarak bu protein aktivitelerini azaltmaktadır. Transkripsiyon faktörleri arasında SOX17 ve SOX18 öne çıkmaktadır, hsa-miR-199a-3p ise bu toksisite ile en önemli mikroRNA'dır. Ayrıca, Ras sinyal yolunun tirozin kinaz inhibitörleri ile ilişkilendirilen kardiyovasküler toksisitelerle çoğunlukla ilişkilendirildiği görülmüştür. Bu bulgular, sunitinib, sorafenib, pazopanib ve axitinib tarafından indüklenen kardiyovasküler hastalıkların altında yatan süreçleri anlamada önemli bir katkı yapmaktadır. Ayrıca, genler, proteinler, transkripsiyon faktörleri, mikroRNA'lar ve yollar da dahil olmak üzere yeni potansiyel terapötik hedefleri ortaya koymaktadır.*

Anahtar Kelimeler: *İn siliko veri madenciliği, kardiyovasküler hastalıklar, tirozin kinaz inhibitörleri*

INTRODUCTION

Tyrosine kinase inhibitors (TKIs) are widely used in clinical practice to treat various cancers, ranging from blood malignancies to advanced solid tumors. Compared to conventional chemotherapy agents such as doxorubicin, TKIs are generally considered safer. However, despite their perceived safety, TKIs are often administered for prolonged periods without a prescribed upper limit on dosage. Consequently, some TKIs still pose significant risks of cardiac adverse events. For example, sunitinib (SUN) has been linked to congestive heart failure in approximately 4.1% of cases in a meta-analysis involving 6935 patients, while sorafenib (SRF) has been shown to cause myocardial ischemia in around 2.7–3% of participants in clinical trials. Additionally, both SUN and SRF are associated with hypertension in up to 47% of patients, likely due to their inhibitory effects on vascular endothelial growth factor (VEGF) signaling [1-5]. A systematic review reported sufficient evidence of high- and all-grade hypertension for pazopanib (PAZ), axitinib (AXI), and SRF. It also indicated probable evidence of all-grade congestive heart failure or left ventricular ejection fraction decline for PAZ and SUN [6]. Another study reported that hypertension was the most commonly reported cardiovascular event and was most frequently associated with SRF and PAZ in 1624 pediatric adverse events linked to TKIs in pediatrics [7]. A case study documented two cases of acute heart failure following PAZ treatment [8]. Another case study reported a patient with metastatic renal cell carcinoma who suddenly developed life-threatening hyperkalemia following the initiation of AXI treatment [9].

TKI-induced cardiotoxicity encompasses two distinct categories: "on-target" and "off-target" effects. Within these categories, cardiac cells exhibit both adaptive and maladaptive reactions to the pharmacological impact of TKIs. Physiological reactions inherent to the heart, such as hypertrophic responses, activation of fetal gene programs, initiation of unfolded protein responses, and stimulation of antioxidant defenses, are frequently triggered by external chemical stressors and may serve to modulate cardiotoxicity adaptively over time. The manifestation of TKI-induced cardiotoxicity is influenced by both the pharmacological inhibition of intended targets or unintended off-targets and the stress responses elicited within cardiac cells [1,10].

To improve the quality of life and clinical treatment of patients with TKI-induced cardiotoxicity, it is necessary to understand the molecular mechanisms of TKI-induced cardiotoxicity. The present study utilized network toxicology strategies to investigate the mechanism of TKI-induced cardiovascular toxicity. Network toxicology is a scientific discipline that employs computer modeling and bioinformatics methods to study the toxic impacts of chemical substances on living organisms. By constructing interaction networks between chemical substances and molecules within organisms,

network toxicology predicts and assesses chemical substance toxicity, aiding in understanding toxicity mechanisms, screening for potentially harmful substances, and more [11]. In addition to network construction, data mining techniques are integral in extracting and analyzing vast datasets to identify patterns and relationships in biological systems relevant to toxicity. Furthermore, *in silico* analysis plays a crucial role by using computational methods to simulate and predict the effects of chemical substances on biological pathways, providing insights into toxic mechanisms and aiding in the identification of biomarkers and therapeutic targets.

The purpose of this research is to gain insight into the toxic mechanisms of TKI-induced cardiotoxicity, elucidate the toxicological profile of four TKIs (SUN, SRF, PAZ, and AXI), and predict their molecular mechanisms. Thus, it provides genomic biomarkers for further *in vitro* and *in vivo* studies.

MATERIAL AND METHOD

Identification of Overlapping Genes Between TKIs and Cardiovascular Diseases

The identification of genes associated with the TKIs (SUN, SRF, PAZ, and AXI) and their connection to cardiovascular diseases (CVDs) was facilitated through the use of the Comparative Toxicogenomics Database (CTD; https://ctdbase.org) and its tools. CTD collects and integrates diverse data on chemical exposures and their biological impacts across different species. This involves manually curating and interconnecting data on chemicals, genes, phenotypes, anatomies, diseases, taxa, and exposures found in published literature [12].

For the analysis of the genes related to the TKIs, each names of TKIs were entered one by one in the "Chemicals" section of the CTD, and all resulting genes were downloaded. The genes associated with CVDs were obtained from the “Direct Evidence” section of CTD, where “M” stands for “marker/mechanism” and “T” stands for “therapeutic.” To obtain common genes related to the four TKIs and CVDs, the Jvenn tool (https://jvenn.toulouse.inrae.fr/app/index.html) was used. It can process up to six lists of input and show the results using either the Edwards-Venn or classical layouts [13]. All findings presented in this study are based on data obtained in April 2024.

Drug-Gene Binary Interaction Analysis

To establish correlations between genes linked to CVDs and genes associated with the TKIs, a manual analysis was conducted using CTD (https://ctdbase.org). This involved scrutinizing the "gene interaction" card in the CTD chemical profile, specifically identifying interactions between the genes and TKIs in terms of protein activity, mRNA expression, and protein expression. The resulting table enumerates the interactions between TKIs and common genes, excluding interactions involving a combination of two or more chemicals and their collective impact on the genes [12].

Protein-Protein Interaction and Centrality Analysis

For protein-protein interactions (PPI) of the common genes between TKIs and CVDs, String v.12.0 (https://string-db.org/cgi) was used [14]. The STRING database systematically collects and integrates protein-protein interactions-both physical interactions and functional associations.

For the analysis, the protein set was entered into the “Multiple Proteins by Names/Identifiers” section, and *Homo sapiens* was selected as the target species. The minimum required interaction score was set to 0.4. The final PPI network was constructed using Cytoscape version 3.10.1 (http://www.cytoscape.org/). Cytoscape is an open-source software for interactive analysis, integration, and visualization of network data [15]. Furthermore, the Molecular Complex Detection (MCODE) algorithm was utilized through Metascape to pinpoint densely interconnected network components. Metascape, accessible via the web at https://metascape.org/gp/index.html#/main/step1, is a web-based platform engineered to deliver thorough annotation and analysis of gene lists. In its design, Metascape integrates functional enrichment, interactome analysis, gene annotation, and membership search functionalities, harnessing the resources of over 40 distinct knowledgebases within a unified portal. Additionally, it streamlines comparative analyses of datasets derived from various independent and orthogonal experiments [16].

Gene Network Analysis

The common genes between TKIs and CVDs, which were put into GeneMANIA (http://genemania.org), were used to study the network of gene-gene interactions. GeneMANIA identifies additional genes associated with a given set of input genes by leveraging an extensive array of functional association data. This dataset encompasses diverse sources such as protein-protein and genetic interactions, pathways, co-expression patterns, co-localization tendencies, and similarities in protein domains. Currently, it maps 166,691 genes from nine organisms [17]. In this study, *Homo sapiens* was chosen as the target organism for analysis, and an automatically selected weighting method was employed.

Analysis of Transcription Factors and MicroRNAs

The common genes between TKIs and CVDs were input into ChIP-X Enrichment Analysis Version 3 (ChEA3) (https://maayanlab.cloud/chea3) to identify the transcription factors (TFs) responsible for their regulation. ChEA3 serves as a web-based tool for analyzing transcription factor (TF) enrichment, organizing TFs linked to gene sets submitted by users. Its background database comprises diverse gene set libraries derived from various origins, encompassing TF-gene coexpression data sourced from RNA-seq investigations, TF-target associations established through ChIP-seq studies, and TF-gene cooccurrence patterns derived from gene lists contributed by the community [18].

Next, the common genes were also subjected to the MIcroRNA Enrichment TURned NETwork (MIENTURNET) tool (http://userver.bio.uniroma1.it/apps/mienturnet/), and *Homo sapiens* was selected as the target to determine potential miRNA networks from miRTarBase that were experimentally confirmed. MIENTURNET uses computationally predicted or experimentally validated miRNA-target interactions from several organisms, including *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Danio rerio* [19]. In the analysis, the threshold for the minimum number of miRNA-target interactions was set at 2, and the adjusted p-value (FDR) was 0.5.

Functional Enrichment Analysis of Common Genes

Gene Ontology (GO) term enrichment analysis was conducted on annotated genes related to TKIs and CVDs using DAVID (https://david.ncifcrf.gov/tools.jsp). The Database for Annotation, Visualization, and Integrated Discovery (DAVID) offers a comprehensive suite of functional annotation resources, enabling researchers to elucidate the biological significance inherent in extensive gene lists [20]. In this analysis, the common gene list was input into DAVID, and *Homo sapiens* was selected as the target species. For gene ontology analysis, the top 5 biological processes, cellular components, and molecular functions were determined, with a p-value < 0.05 and false discovery rate (FDR) < 0.05 set as the cutoff criteria.

For molecular pathways analysis, Metascape was employed [16]. In this analysis, the common gene list was input into Metascape, and *Homo sapiens* was selected as the target species. The significance of the results was determined by p and q values. P-values were computed utilizing the cumulative hypergeometric distribution, and q-values were determined employing the Benjamini-Hochberg method to control the false discovery rate (FDR) via sequential modified Bonferroni correction for multiple hypothesis testing, thereby addressing the issue of multiple comparisons [21].

RESULT AND DISCUSSION

Common Genes Associated with TKIs and CVDs

Searching the CTD database showed that SUN, SRF, PAZ, and AXI target 6220, 196, 24, and 11 genes, respectively. Additionally, the number of CVD-associated genes was 3.79 million; 1674 of them were marked as “markers/mechanisms” and/or “therapeutics” in the "Direct Evidence" section. Six genes were common between the four TKIs and CVDs, alphabetically: Cytochrome P450 3A4 (CYP3A4), Mitogen-activated protein kinase 1 (MAPK1, ERK2), Mitogen-activated protein kinase (MAPK3), Vascular endothelial growth factor receptor 1 (FLT1, VEGFR1), Vascular endothelial

growth factor receptor 2 (KDR, VEGFR2), and Vascular endothelial growth factor receptor 3 (FLT4, VEGFR3) (Figure 1B).

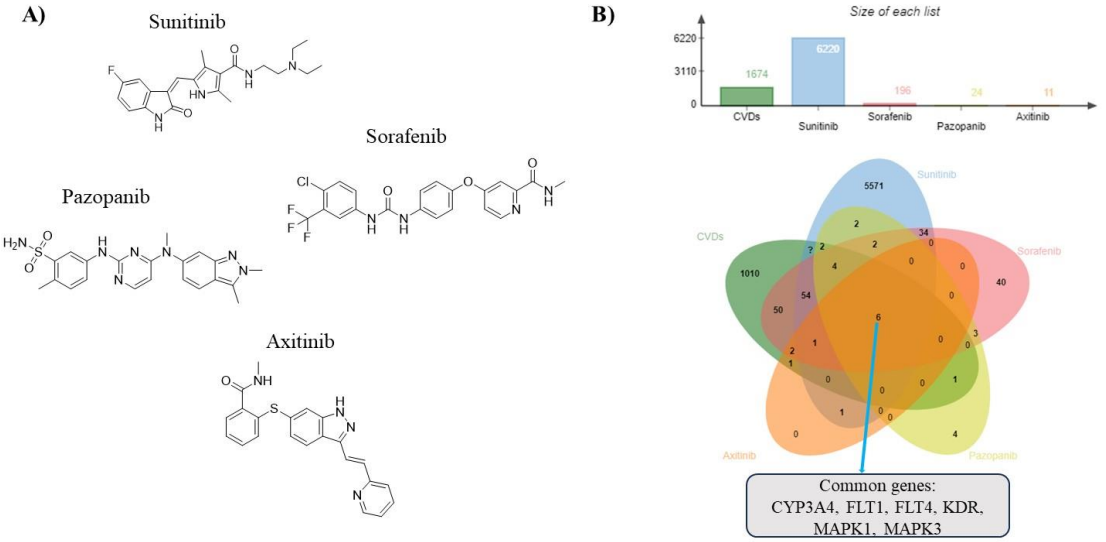

Figure 1. A) Tyrosine kinase inhibitors (TKIs) often associated with cardiovascular toxicity, **B)** Common genes between TKIs and CVDs

TKIs-Gene Binary Interaction Analysis Results

Individual chemical-gene interactions were analyzed to uncover potential overlaps in protein activity, protein expression, and mRNA expression (Table 1). The findings suggest that the examined TKIs can downregulate the activity, mRNA expression, and protein expression of the five hub genes. Given that TKIs also target these hub genes in cancer cells and reduce their activity and/or expression, their ability to downregulate these genes in the cardiovascular system may indicate on-target toxicity.

Table 1. TKIs-gene binary analysis results

	FLT1 (VEGFR1)			**FLT4** (VEGFR3)			**KDR** (VEGFR2)			**MAPK1** (ERK2)			**MAPK3**		
TKIs	P.A.	mR. E.	P. E.	P. A.	mR. E.	P. E.	P. A.	mR. E.	P. E.	P. A.	mR. E.	P. E	P. A.	mR. E.	P. E.
SUN	↓	↓		↓		↓	↓	↓		↓			↓		
SRF	↓			↓			↓			↓		↓	↓		
PAZ	↓						↓			↓			↓		
AXI	↓			↓			↓			↓			↓		

P. A. = Protein activitiy. mR. E. = mRNA expression. P. E. = Protein expression

PPI, Centrality Analysis, and Gene-Gene Network

The PPI network analysis depicted 6 nodes and 7 edges, as shown in Figure 2A (upper panel), with a significant PPI enrichment p-value of 0.00263. Additionally, centrality analysis was conducted to identify hub proteins, revealing five hub proteins associated with TKIs-induced CVDs (Figure 2A, lower panel).

To construct a connected network from shared genes, the GeneMANIA online plug-in was utilized. The findings indicated that a majority of genes linked to the examined TKIs were involved in physical interactions (77.64%), whereas other interaction types were less prominent (co-expression (8.01%); predicted interactions (5.37%); colocalization (3.63%); genetic interactions (2.87%); pathway (1.88%); shared protein domains (0.60%) (Figure 2B). These outcomes emphasize the prevalence of physical interactions among hub genes associated with CVDs, highlighting the pivotal role of direct molecular associations in the pathogenesis induced by TKIs.

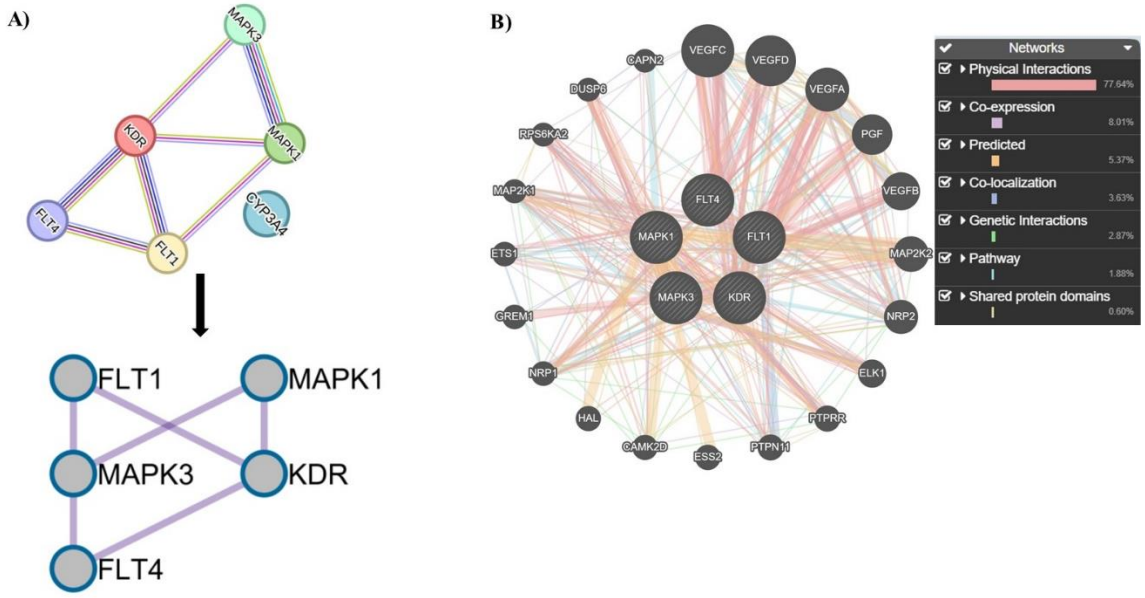

Figure 2. A) PPI analysis of common genes and hub genes, **B)** Gen-gen interaction analysis of the hub genes

Core Transcription Factors and miRNAs Involved in CVDs Induced by TKIs

After analyzing transcription factors (TFs) for five hub genes in ChEA3, the top 10 TFs were identified. Subsequently, nodes and edges representing the relationships between TFs and hub genes were manually prepared in Excel and schematized in Cytoscape 3.10.1, as shown in Figure 3A. The results are sorted by mean rank (Table 2). 'MeanRank' refers to the mean rank of each TF across all libraries containing that TF, serving as the score by which a composite list of TFs is reranked. In Figure 3A, green nodes represent TFs, whereas light brown nodes represent hub genes. These TFs may serve as potential targets for treating patients with TKIs-induced CVDs. Although there have not been any studies on some Table 2 TFs, there are studies highlighting the relationship between these TFs and CVDs. For instance, it is suggested that genetic variations in SOX18 and SOX17 in humans contribute to congenital heart disease [22,23]. Another study reported that SOX7 deficiency causes ventricular septal defects [24]. Other TFs, such as HAND1, play an essential role in cardiac morphogenesis [25].

Table 2. The top 10 TFs associated with to five hub genes

Rank	TF	Mean Rank	Overlapping Genes
1	BCL6B	2.667	FLT1, FLT4, KDR
2	SOX18	3.667	FLT1, FLT4, KDR
3	SOX17	28.25	FLT1, FLT4, KDR
4	HAND1	35.0	FLT1, KDR
5	SOX7	45.0	FLT1, FLT4, KDR
6	FOXE1	51.67	FLT1, FLT4, KDR
7	GSC	54.0	FLT1, KDR
8	SALL4	62.5	FLT4, KDR, MAPK1
9	ANHX	100.0	KDR
10	LHX1	101.0	KDR

As a result of miRNA-target analysis, the top 10 miRNAs were identified and are shown in Figure 3B. Among these 10 miRNAs, hsa-miR-199a-3p emerged as the most important miRNA in TKIs-induced CVDs. Some studies suggest that hsa-miR-199a-3p might be beneficial. For instance, Joris et al. [26] reported that it could help with repair after a heart attack by promoting heart muscle cell growth.

This is according to a study highlighting the role of hsa-miR-199a-3p in regulating endothelial nitric oxide synthase pathway, which is crucial for blood vessel health. Eulalio et al., [27] reported that hsa-miR-199a stimulated marked cardiac regeneration and almost complete recovery of cardiac functional parameters after myocardial infarction in mice.

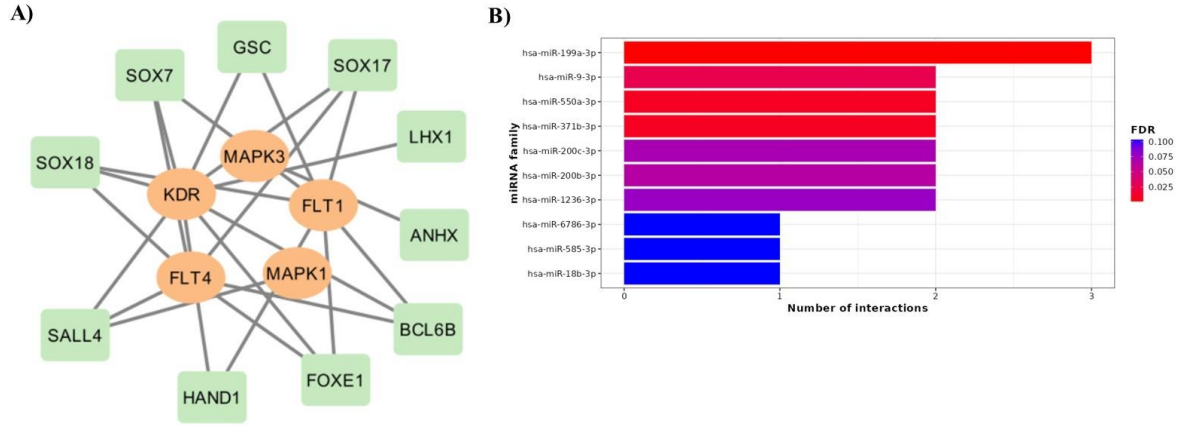

Figure 3. A) Core transcription factors associated with five hub genes obtained from ChEA3 and prepared in Cytoscape, **B)** Core miRNAs associated with five hub genes obtained from MIENTURNET

GO Enrichment Analyses and Molecular Pathways of Hub Genes

The top five enriched terms associated with TKIs-induced CVDs, identified in each GO category (biological processes, cellular components, and molecular functions) using the DAVID web tool, are outlined in Table 3. The findings underscore phosphorylation, a fundamental cellular process involving the addition of phosphate groups to proteins, as a primary biological process associated with hub genes. This suggests that dysregulation of phosphorylation pathways may play a pivotal role in the pathogenesis of CVDs resulting from exposure to SUN, SRF, PAZ, and AXI.

Furthermore, the analysis identified the receptor complex, a dynamic assembly of proteins that mediate cellular signaling and responses to extracellular stimuli, as the most significant cellular component involved in these four TKI-induced CVDs (Table 3). This highlights the importance of receptor-mediated signaling pathways in the development and progression of CVDs associated with TKI exposure.

Moreover, vascular endothelial growth factor-activated receptor activity emerged as the predominant molecular function implicated in these four TKI-induced CVDs, underscoring the critical role of VEGF signaling in vascular homeostasis and endothelial function. Dysregulation of VEGF receptor activity may disrupt angiogenesis and vascular integrity, contributing to the pathophysiology of TKI-induced CVDs. These insights shed light on the molecular mechanisms underlying the adverse cardiovascular effects of TKIs and may inform strategies for mitigating their cardiotoxicity (Table 3).

The molecular pathway associated with the five hub genes determined via Metascape highlighted 'Ras signaling' as the top pathway, with Log10(p) value of -11.11 and Log10(q) value of -7.22 (Figure 4 and 5).

Ras signaling is a crucial pathway involved in regulating various cellular processes, including cell growth, differentiation, and survival. The Ras protein acts as a molecular switch, cycling between an active (GTP-bound) and inactive (GDP-bound) state, and plays a central role in transmitting extracellular signals to the nucleus, thereby influencing gene expression and cellular behavior. The central role of Ras in pathologic and physiologic cardiac hypertrophy has been demonstrated in the literature. The dysregulation of Ras signaling has been implicated in several pathological conditions, including hypertrophy, fibrosis, and cardiomyopathy [28-31]. Activation of Ras signaling can lead to cardiac hypertrophy, a condition characterized by an increase in the size of individual cardiac muscle

cells. This is often a compensatory response to increased workload or pathological stimuli, such as hypertension or myocardial infarction. Prolonged hypertrophy can ultimately lead to heart failure. Ras signaling has been linked to the activation of fibroblasts and the production of extracellular matrix proteins, contributing to cardiac fibrosis. Fibrosis is the excessive deposition of collagen and other matrix components in the myocardium, leading to stiffening of the heart muscle and impaired function [28-31]. Given the role of Ras signaling in TKIs-induced cardiovascular pathology, it has emerged as a potential therapeutic target for the treatment of various CVDs. Strategies aimed at modulating Ras activity or downstream effectors may offer new avenues for intervention in conditions such as heart failure, myocardial infarction, and cardiac hypertrophy induced by TKIs.

Table 3. The top 5 gene ontology enrichments, include biological processes, cellular components, and molecular functions associated with the five hub genes (https://david.ncifcrf.gov/tools.jsp)

No.	ID	Biological Processes	*P* value	Bonferroni	Benjamini	FDR	Fisher Exact
1	GO:0016310	phosphorylation	$5.3x10^{-6}$	$9.6x10^{-4}$	$7.5x10^{-4}$	$5.4x10^{-4}$	$2.0x10^{-7}$
2	GO:0038084	vascular endothelial growth factor signaling pathway	$8.2x10^{-6}$	$1.5x10^{-3}$	$7.5x10^{-4}$	$5.4x10^{-4}$	$1.4x10^{-8}$
3	GO:0048010	vascular endothelial growth factor receptor signaling pathway	$2.3x10^{-5}$	$4.2x10^{-3}$	$1.3x10^{-3}$	$9.4x10^{-4}$	$6.8x10^{-8}$
4	GO:0035924	cellular response to vascular endothelial growth factor stimulus	$2.8x10^{-5}$	$5.2x10^{-3}$	$1.3x10^{-3}$	$9.4x10^{-4}$	$9.1x10^{-8}$
5	GO:0080090	regulation of primary metabolic process	$4.6x10^{-5}$	$8.4x10^{-3}$	$1.7x10^{-3}$	$1.2x10^{-3}$	$1.9x10^{-7}$
		Cellular Component					
1	GO:0043235	receptor complex	$1.0x10^{-3}$	$3.5x10^{-2}$	$3.6x10^{-2}$	$3.1x10^{-2}$	$2.1x10^{-5}$
2	GO:0005769	early endosome	$2.2x10^{-3}$	$7.5x10^{-2}$	$3.8x10^{-2}$	$3.3x10^{-2}$	$6.7x10^{-5}$
3	GO:0005925	focal adhesion	$4.2x10^{-3}$	$1.4x10^{-1}$	$3.8x10^{-2}$	$3.3x10^{-2}$	$1.7x10^{-4}$
4	GO:0031143	pseudopodium	$4.4x10^{-3}$	$1.4x10^{-1}$	$3.8x10^{-2}$	$3.3x10^{-2}$	$1.1x10^{-5}$
5	GO:0005901	caveola	$1.8x10^{-2}$	$4.7x10^{-1}$	$1.1x10^{-1}$	$9.4x10^{-2}$	$2.0x10^{-4}$
		Molecular Function					
1	GO:0005021	vascular endothelial growth factor-activated receptor activity	$1.6x10^{-6}$	$6.7x10^{-5}$	$6.7x10^{-5}$	$6.0x10^{-5}$	$1.0x10^{-9}$
2	GO:0019838	growth factor binding	$4.1x10^{-5}$	$1.8x10^{-3}$	$6.9x10^{-4}$	$6.1x10^{-4}$	$1.6x10^{-7}$
3	GO:0004714	transmembrane receptor protein tyrosine kinase activity	$4.8x10^{-5}$	$2.1x10^{-3}$	$6.9x10^{-4}$	$6.1x10^{-4}$	$2.0x10^{-7}$
4	GO:0005524	ATP binding	$2.1x10^{-4}$	$8.8x10^{-3}$	$2.2x10^{-3}$	$2.0x10^{-3}$	$2.0x10^{-5}$
5	GO:0004713	protein tyrosine kinase activity	$3.5x10^{-4}$	$1.5x10^{-2}$	$3.0x10^{-3}$	$2.7x10^{-3}$	$4.1x10^{-6}$

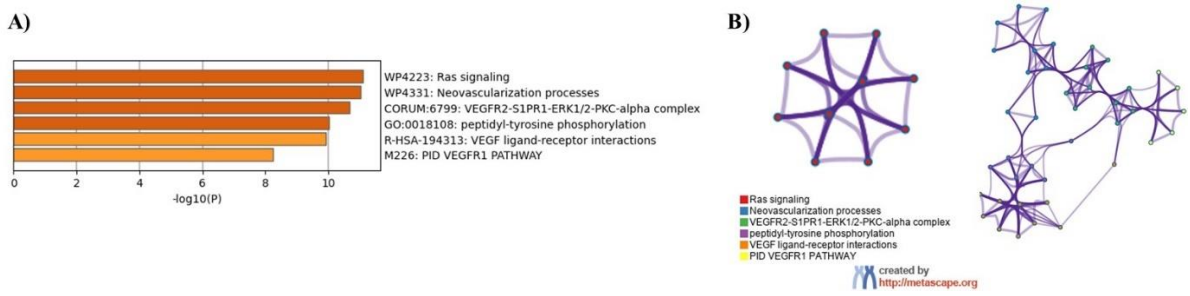

Figure 4. A) The top molecular pathways associated with five hub genes obtained from Metascape, **B)** The pathways associated with five hub genes are colored by its *p*-value obtained from Metascape

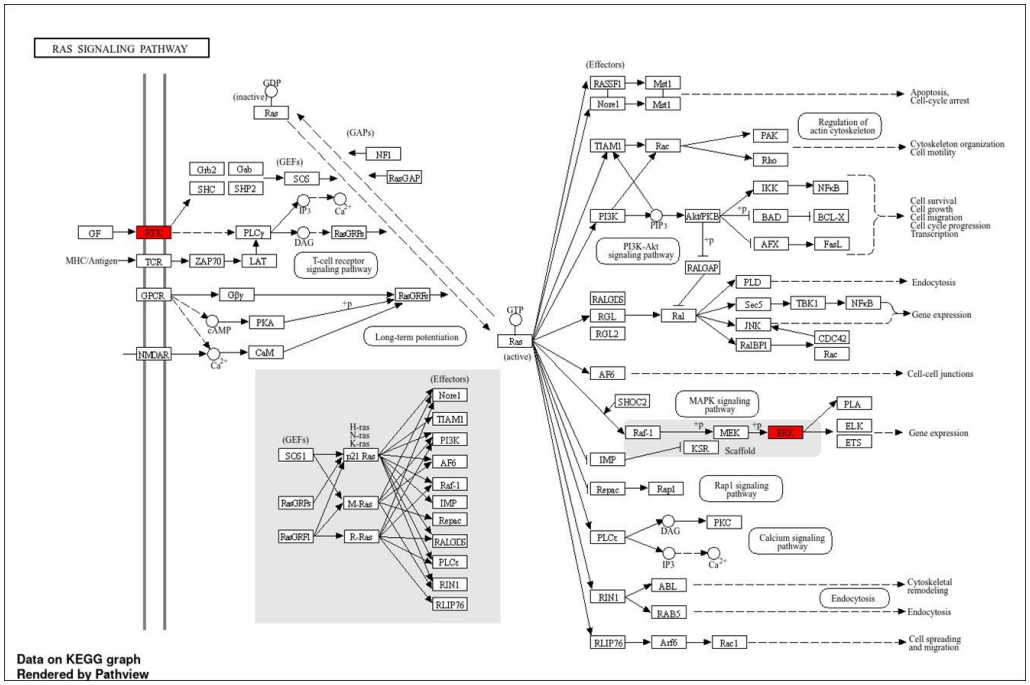

Figure 5. Ras signaling pathway downloaded from KEGG pathway (https://www.genome.jp/kegg/) [32]

In conclusion, this study employed network toxicology and bioinformatics approaches to elucidate the molecular mechanisms underlying cardiovascular toxicity induced by TKIs such as SUN, SRF, PAZ, and AXI. Through comprehensive data mining and analysis, we identified FLT1, FLT4, KDR, MAPK1, and MAPK3 as hub genes/proteins, SOX17 and SOX18 as transcription factors, and hsa-miR-199a-3p as a key microRNA, along with pathways implicated in TKI-induced cardiotoxicity. Notably, Ras signaling emerged as a pivotal pathway in mediating these adverse effects. These findings not only enhance our understanding of TKI-associated cardiovascular diseases but also highlight potential targets for future therapeutic interventions aimed at mitigating TKI-induced cardiotoxicity and improving patient outcomes.

AUTHOR CONTRIBUTIONS

Concept: F.K.; Design: F.K.; Control: F.K.; Sources: F.K.; Materials: F.K.; Data Collection and/or Processing: F.K.; Analysis and/or Interpretation: F.K.; Literature Review: F.K.; Manuscript Writing: F.K.; Critical Review: F.K.; Other: -

CONFLICT OF INTEREST

The author declares that there are no actual, potential or perceived conflicts of interest.

ETHICS COMMITTEE APPROVAL

The author declares that ethics committee approval is not required for this study.

REFERENCES

1. Wang, H., Wang, Y., Li, J., He, Z., Boswell, S.A., Chung, M., You, F., Han, S. (2023). Three tyrosine kinase inhibitors cause cardiotoxicity by inducing endoplasmic reticulum stress and inflammation in cardiomyocytes. BMC Medicine, 21(1), 147. [CrossRef]
2. Richards, C.J., Je, Y., Schutz, F.A., Heng, D.Y., Dallabrida, S.M., Moslehi, J.J., Choueiri, T.K. (2011). Incidence and risk of congestive heart failure in patients with renal and nonrenal cell carcinoma treated

with sunitinib. Journal of Clinical Oncology: Official Journal of The American Society of Clinical Oncology, 29(25), 3450-3456. [CrossRef]
3. Escudier, B., Eisen, T., Stadler, W.M., Szczylik, C., Oudard, S., Siebels, M., Negrier, S., Chevreau, C., Solska, E., Desai, A.A., Rolland, F., Demkow, T., Hutson, T.E., Gore, M., Freeman, S., Schwartz, B., Shan, M., Simantov, R., Bukowski, R.M., TARGET Study Group. (2007). Sorafenib in advanced clear-cell renal-cell carcinoma. The New England Journal of Medicine, 356(2), 125-134. [CrossRef]
4. Llovet, J.M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J.F., de Oliveira, A.C., Santoro, A., Raoul, J.L., Forner, A., Schwartz, M., Porta, C., Zeuzem, S., Bolondi, L., Greten, T.F., Galle, P.R., Seitz, J.F., Borbath, I., Häussinger, D., Giannaris, T., Shan, M., Moscovici, M., Voliotis, D., Bruix, J. (2008). Sorafenib in advanced hepatocellular carcinoma. The New England Journal of Medicine, 359(4), 378-390. [CrossRef]
5. Chu, T.F., Rupnick, M.A., Kerkela, R., Dallabrida, S.M., Zurakowski, D., Nguyen, L., Woulfe, K., Pravda, E., Cassiola, F., Desai, J., George, S., Morgan, J.A., Harris, D.M., Ismail, N.S., Chen, J.H., Schoen, F.J., Van den Abbeele, A.D., Demetri, G.D., Force, T., Chen, M.H. (2007). Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. Lancet, 370(9604), 2011-2019. [CrossRef]
6. Van Leeuwen, M.T., Luu, S., Gurney, H., Brown, M.R., Pearson, S.A., Webber, K., Hunt, L., Hong, S., Delaney, G.P., Vajdic, C.M. (2020). Cardiovascular Toxicity of Targeted Therapies for Cancer: An Overview of Systematic Reviews. JNCI Cancer Spectrum, 4(6), pkaa076. [CrossRef]
7. Narayan, H.K., Sheline, K., Wong, V., Kuo, D., Choo, S., Yoon, J., Leger, K., Kutty, S., Fradley, M., Tremoulet, A., Ky, B., Armenian, S., Guha, A. (2023). Cardiovascular toxicities with pediatric tyrosine kinase inhibitor therapy: An analysis of adverse events reported to the Food and Drug Administration. Pediatric Blood & Cancer, 70(2), e30059. [CrossRef]
8. Kertmen, N., Kavgaci, G., Yildirim, H.C., Dizdar, O. (2024). Acute heart failure following pazopanib treatment: a literature review featuring two case reports. *Anti-Cancer Drugs*, 35(3), 302-304. [CrossRef]
9. Godo, S., Yoshida, Y., Kawamorita, N., Mitsuzuka, K., Kawazoe, Y., Fujita, M., Kudo, D., Nomura, R., Shimokawa, H., Kushimoto, S. (2018). Life-threatening Hyperkalemia Associated with Axitinib Treatment in Patients with Recurrent Renal Carcinoma. Internal Medicine (Tokyo, Japan), 57(19), 2895-2900. [CrossRef]
10. Chen, M.H., Kerkelä, R., Force, T. (2008). Mechanisms of cardiac dysfunction associated with tyrosine kinase inhibitor cancer therapeutics. Circulation, 118(1), 84-95. [CrossRef]
11. Zhang W. (2018). Fundamentals of network biology. Chapter 21: Network Pharmacology and Toxicology, World Scientific, pp. 391-411. [CrossRef]
12. Davis, A.P., Wiegers, T.C., Johnson, R.J., Sciaky, D., Wiegers, J., Mattingly, C.J. (2023). Comparative Toxicogenomics Database (CTD): update 2023. Nucleic Acids Research, 51(D1), D1257-D1262. [CrossRef]
13. Bardou, P., Mariette, J., Escudié, F., Djemiel, C., Klopp, C. (2014). jvenn: an interactive Venn diagram viewer. BMC Bioinformatics, 15(1), 293. [CrossRef]
14. Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A.L., Fang, T., Doncheva, N.T., Pyysalo, S., Bork, P., Jensen, L.J., von Mering, C. (2023). The STRING database in 2023: Protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Research, 51(D1), D638-D646. [CrossRef]
15. Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T. (2003). Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Research, 13(11), 2498-2504. [CrossRef]
16. Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A.H., Tanaseichuk, O., Benner, C., Chanda, S.K. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nature Communications, 10(1), 1523. [CrossRef]
17. Warde-Farley, D., Donaldson, S.L., Comes, O., Zuberi, K., Badrawi, R., Chao, P., Franz, M., Grouios, C., Kazi, F., Lopes, C.T., Maitland, A., Mostafavi, S., Montojo, J., Shao, Q., Wright, G., Bader, G.D., Morris, Q. (2010). The GeneMANIA prediction server: Biological network integration for gene prioritization and predicting gene function. Nucleic Acids Research, 38(Web Server issue), W214-W220. [CrossRef]
18. Keenan, A.B., Torre, D., Lachmann, A., Leong, A.K., Wojciechowicz, M.L., Utti, V., Jagodnik, K.M., Kropiwnicki, E., Wang, Z., Ma'ayan, A. (2019). ChEA3: Transcription factor enrichment analysis by orthogonal omics integration. Nucleic Acids Research, 47(W1), W212-W224. [CrossRef]
19. Licursi, V., Conte, F., Fiscon, G., Paci, P. (2019). MIENTURNET: An interactive web tool for microRNA-target enrichment and network-based analysis. BMC Bioinformatics, 20(1), 545. [CrossRef]

20. Sherman, B.T., Hao, M., Qiu, J., Jiao, X., Baseler, M.W., Lane, H.C., Imamichi, T., Chang, W. (2022). DAVID: A web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Research, 50(W1), W216-W221. [CrossRef]
21. Benjamini, Y., Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of The Royal Statistical Society: Series B (Methodological), 57(1), 289-300. [CrossRef]
22. Shi, H.Y., Xie, M.S., Yang, C.X., Huang, R.T., Xue, S., Liu, X.Y., Xu, Y.J., Yang, Y.Q. (2022). Identification of SOX18 as a New Gene Predisposing to Congenital Heart Disease. Diagnostics (Basel, Switzerland), 12(8), 1917. [CrossRef]
23. Zhao, L., Jiang, W.F., Yang, C.X., Qiao, Q., Xu, Y.J., Shi, H.Y., Qiu, X.B., Wu, S.H., Yang, Y.Q. (2021). SOX17 loss-of-function variation underlying familial congenital heart disease. European Journal of Medical Genetics, 64(5), 104211. [CrossRef]
24. Hernández-García, A., Pendleton, K.E., Kim, S., Li, Y., Kim, B.J., Zaveri, H.P., Jordan, V.K., Berry, A. M., Ljungberg, M.C., Chen, R., Lanz, R.B., Scott, D.A. (2023). SOX7 deficiency causes ventricular septal defects through its effects on endocardial-to-mesenchymal transition and the expression of Wnt4 and Bmp2. Human Molecular Genetics, 32(13), 2152-2161. [CrossRef]
25. Knöfler, M., Meinhardt, G., Vasicek, R., Husslein, P., Egarter, C. (1998). Molecular cloning of the human Hand1 gene/cDNA and its tissue-restricted expression in cytotrophoblastic cells and heart. Gene, 224(1-2), 77–86. [CrossRef]
26. Joris, V., Gomez, E.L., Menchi, L., Lobysheva, I., Di Mauro, V., Esfahani, H., Condorelli, G., Balligand, J.L., Catalucci, D., Dessy, C. (2018). MicroRNA-199a-3p and MicroRNA-199a-5p take part to a redundant network of regulation of the NOS (NO Synthase)/NO pathway in the endothelium. Arteriosclerosis, Thrombosis, and Vascular Biology, 38(10), 2345-2357. [CrossRef]
27. Eulalio, A., Mano, M., Dal Ferro, M., Zentilin, L., Sinagra, G., Zacchigna, S., Giacca, M. (2012). Functional screening identifies miRNAs inducing cardiac regeneration. Nature, 492(7429), 376-381. [CrossRef]
28. Ramos-Kuri, M., Rapti, K., Mehel, H., Zhang, S., Dhandapany, P.S., Liang, L., García-Carrancá, A., Bobe, R., Fischmeister, R., Adnot, S., Lebeche, D., Hajjar, R.J., Lipskaia, L., Chemaly, E.R. (2015). Dominant negative Ras attenuates pathological ventricular remodeling in pressure overload cardiac hypertrophy. Biochimica et Biophysica Acta, 1853(11 Pt A), 2870-2884. [CrossRef]
29. Zhao, C.Z., Zhao, X.M., Yang, J., Mou, Y., Chen, B., Wu, H.D., Dai, D.P., Ding, J., Hu, S.J. (2016). Inhibition of farnesyl pyrophosphate synthase improves pressure overload induced chronic cardiac remodeling. Scientific Reports, 6, 39186. [CrossRef]
30. Linglart, L., Gelb, B.D. (2020). Congenital heart defects in Noonan syndrome: Diagnosis, management, and treatment. American Journal of Medical Genetics. Part C, Seminars in Medical Genetics, 184(1), 73-80. [CrossRef]
31. Ramos-Kuri, M., Meka, S.H., Salamanca-Buentello, F., Hajjar, R.J., Lipskaia, L., Chemaly, E.R. (2021). Molecules linked to Ras signaling as therapeutic targets in cardiac pathologies. Biological Research, 54(1), 23. [CrossRef]
32. Kanehisa, M., Furumichi, M., Sato, Y., Kawashima, M., Ishiguro-Watanabe, M. (2023). KEGG for taxonomy-based analysis of pathways and genomes. Nucleic Acids Research, 51(D1), D587-D592. [CrossRef]