Acta Biochimica Indonesiana

https://doi.org/10.32889/actabioina.110

RESEARCH ARTICLE

Open Access

Identification of cardiac premature senescence markers through GEO database interaction approach

Tiwuk Susantiningsih[1,2], Ani Retno Prijanti[3], Novi Silvia Hardiany[3], Fadilah[4]

[1]Doctoral Program of Biomedical Science, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia
[2]Department of Biochemistry Faculty of Medicine, UPN Veteran Jakarta, Jakarta, Indonesia
[3]Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia
[4]Department of Medical Chemistry, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia
*Corresponding author: Doctoral Program of Biomedical Science, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia.
Email: tiwuksusantiningsih@gmail.com

ABSTRACT

Background: Cardiomyocytes, the muscle cells of the heart, susceptible to premature senescence, a condition contributing to various cardiovascular diseases. However, there is a limited amount of in silico research on premature senescence in cardiomyocytes.

Purpose: This study aims to identify key markers of premature cardiac senescence using an in silico approach through the GEO database.

Methods: Protein data was obtained from the GeneCards database using the keywords: premature senescence, cardiovascular, cardiomyocytes, and p53. The identified proteins were analyzed using Cytoscape 3.9.1 and StringDB to assess protein-protein interactions and determine the most significant proteins involved in cardiac premature senescence.

Results: The search yielded 1,046 proteins, which were narrowed down to the top 100 using Cytoscape analysis. Further analysis with StringDB identified 16 key proteins. Among these, TP53, CDK2, and PTEN were found to have the highest interaction scores.

Conclusion: The integration of GeneCards, Cytoscape, and StringDB data revealed that TP53, CDK2, and PTEN are essential markers of premature cardiac senescence. These findings provide valuable insights into the molecular mechanisms of cardiac aging and potential therapeutic targets for cardiovascular diseases.

Keywords: premature cardiac senescence, p53, GEO database

Introduction

Cardiovascular disease is a leading cause of death globally, accounting for 30% of deaths according to the World Health Organization (WHO) [1]. Heart attacks and strokes are significant contributors, with 7.6 million and 5.7 million cases, respectively. Premature senescence, a condition of permanent cell cycle arrest due to DNA damage and/or mitochondrial damage [2–5], is a critical factor in the pathology of cardiovascular disease.

Premature senescence can occur in cardiac cells due to various triggers. It is primarily mediated through the p53 or p16 signaling pathways [6]. This process is characterized by irreversible cell cycle arrest, DNA damage, changes in metabolic activity, and the secretion of a complex mix of proinflammatory cytokines, chemokines, growth factors, and proteolytic enzymes known as the senescence-associated secretory phenotype (SASP) [7,8]. Cells undergoing premature senescence become flattened, enlarged, and lose the ability to proliferate permanently [9].

Several factors can induce premature senescence independently of telomere shortening [10], including exposure to cigarette smoke, ultraviolet light,

irradiation, chemotherapy, and oncogenic signaling [3]. It can also be induced experimentally in vitro or in vivo through DNA damaging agents and oxidative stress [11]. Research by Anderson et al. demonstrated that senescence can occur in post-mitotic cells, such as cardiomyocytes and neurons, independent of cell proliferation [12].

When DNA and/or mitochondrial damage occurs, cells exit the G1 phase into a temporary growth arrest (TGA) stage for repair. If the damage is irreparable, cells either undergo premature senescence, apoptosis, or autophagy [13–15]. Proteins such as p53 and p16, which act as strong tumor suppressors, play crucial roles in this process by restraining cell proliferation. However, this process also carries the risk of transforming into malignant cells, such as cancer. Aging cells that exit the cell cycle permanently can have detrimental effects on surrounding tissues.

Premature senescence can occur in the heart for various reasons [16–18], particularly in cardiomyocytes, the muscle cells of the heart. Despite its importance, there is limited in silico research on premature senescence in cardiomyocytes. This study aims to identify markers of cardiac premature senescence using the GEO database interaction approach. Identifying these markers can provide insights into the mechanisms of cardiac aging and potential therapeutic targets for preventing cardiovascular diseases.

Method

The research commenced with the use of the GeneCards website (https://www.genecards.org/) to obtain proteins related to keywords. Four keywords associated with cardiac premature senescence were used, including premature senescence, cardiovascular, cardiomyocytes, and p53. The identified proteins were then analyzed using Cytoscape Tools version 3.9.1 to assess the interaction scores between the proteins. The criteria from the website included a required score, with the highest confidence set at 0.900 and high FDR stringency at 1%.

Protein-protein interaction

Protein-protein interactions (PPI) play a crucial role in cell regulation and signaling. The proteins with the highest interaction scores were identified, and their connections were analyzed using StringDB (https://string-db.org/cgi/input?sessionId=bOLkcyWFsnPH&input_page_active_form=multiple_identifiers). The analyses from StringDB and Cytoscape facilitated the identification of cardiac premature senescence markers through the GEO database interaction approach.

Results

Gene database on cardiac premature senescence

Protein data was obtained from the GeneCards database using four keywords: premature senescence, cardiovascular, cardiomyocytes, and p53. This search yielded 1,046 proteins with the highest rankings. The data was exported from GeneCards, and the top 100 proteins, with scores ranging from 161.98 to 19.18, were selected, including ACTA2 and TP53 proteins.

The analysis proceeded to examine protein-protein interactions using Cytoscape 3.9.1. Utilizing the String protein query with a confidence score cutoff of 0.90, 100 protein identifiers from GeneCards were analyzed. The data from Cytoscape was exported from the edge table and sorted by value and hierarchy based on four criteria: the values of stringdb:score, stringdb:coexpression, stringdb:experiments, and stringdb:textmining. Among the 100 protein identifiers with stringdb:score value of 999, 56 protein interactions were identified. The top 10 protein interactions with the highest scores are listed in Table 1.

Table 1 shows that the protein interactions with the highest scores are CDK2 and PCNA. These proteins were further analyzed using StringDB. The results of this analysis are presented in Figure 1.

Table 1. The highest scores of 10 proteins of the protein protein interaction data

No	Protein interaction	Stringdb: score	Stringdb: coexpression	Stringdb: experiments	Stringdb: text mining
1	CDK2 → PCNA	999	685	992	485
2	RB1 → E2F1	999	145	999	99
3	CHEK2 → BRCA1	999	132	879	955
4	SIRT1 → EP300	999	99	733	98
5	FGF2 → FGFR1	999	98	957	991
6	CDK2 → TP53	999	95	791	97
7	PIK3CA → PIK3R1	999	84	999	991
8	MTOR → AKT1	999	82	905	991
9	TP53 → BRCA1	999	77	9	99
10	PTEN → PIK3R1	999	65	342	99

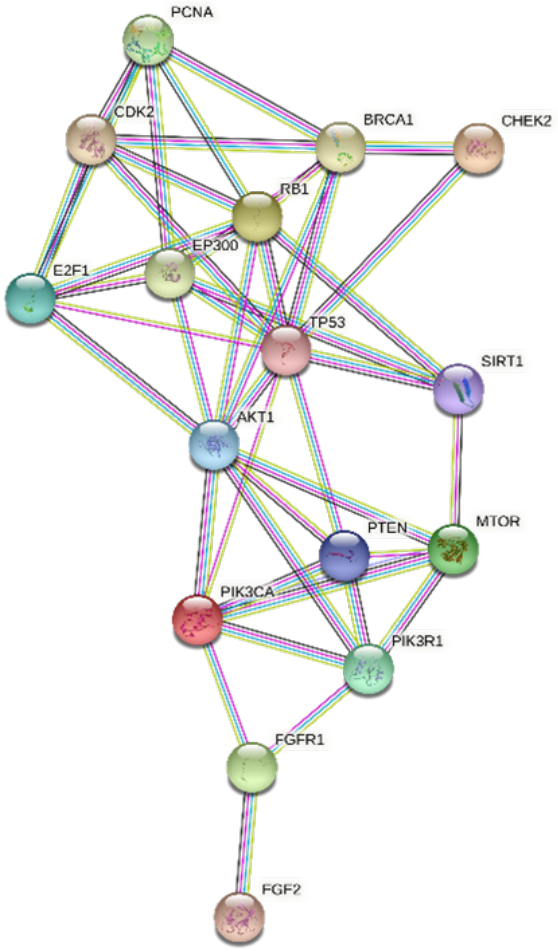

Figure 1. Graph of network of StringDB protein query protein protein interaction

Protein-protein interactions in cell regulation and signaling

The p53 protein, encoded by a gene located on chromosome 17: 7,661,779-7,687,550 on the reverse strand, acts as a tumor suppressor in many types of tumors. Depending on the physiological state and cell type, p53 can induce growth arrest or apoptosis. It is also involved in cell cycle regulation as a trans-activator, negatively controlling cell division by regulating a set of genes required for this process. One of the activated genes is cyclin-dependent kinase inhibitor (CKI). Induction of apoptosis by p53 is mediated through the stimulation of BAX and FAS antigen expression or the repression of Bcl-2 expression.

The p53 gene encodes a tumor suppressor protein with transcriptional activation, DNA binding, and oligomerization domains. The TP53-encoded protein responds to various cellular stresses to regulate target gene expression, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or metabolic changes. Mutations in the p53 gene are associated with various human cancers.

Jangid et al. studied a minimal model of the stress-driven p53 regulatory network, including competition between active and mutant forms of the p53 tumor suppressor gene [19]. Based on the characteristics and level of the external voltage signal, four different dynamic states of p53 are observed: active, apoptotic, pre-malignant, and cancer. The

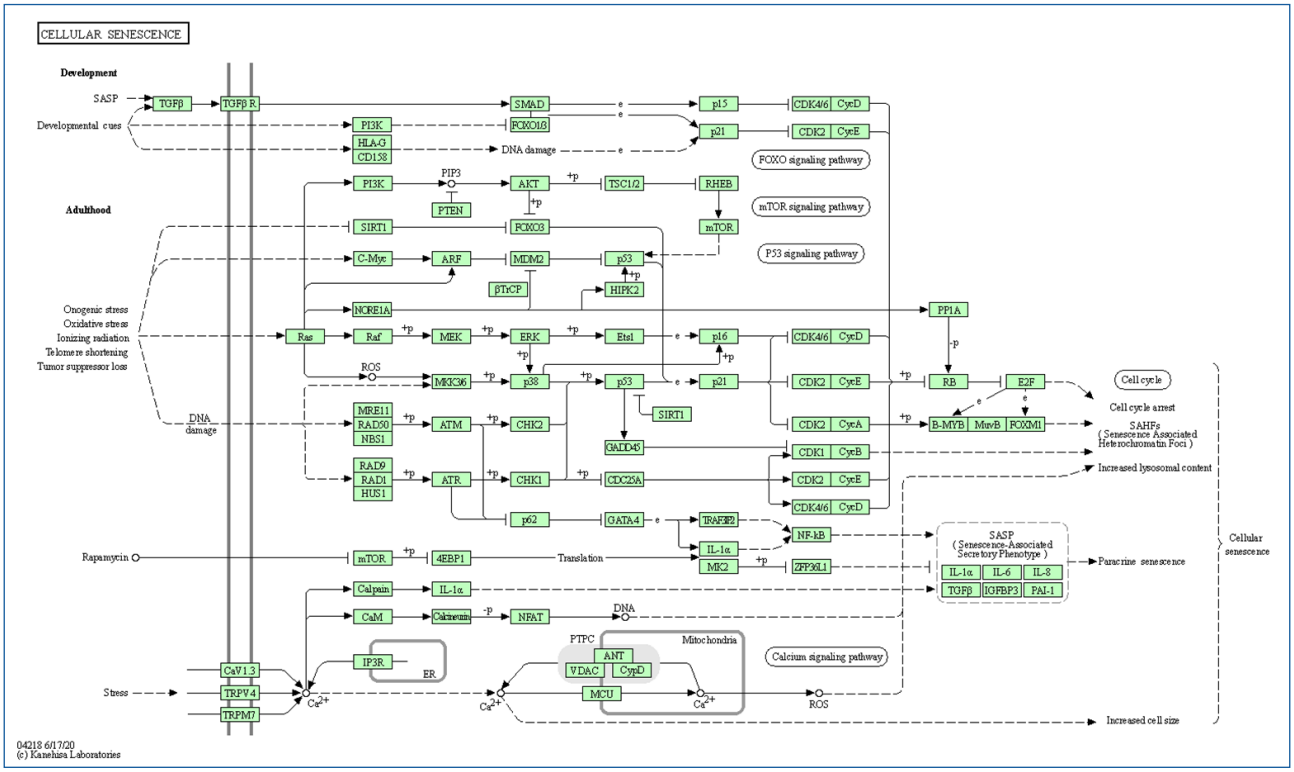

Figure 2. KEGG pathway cellular senescence.

transition between these states was found to be unidirectional and irreversible if the stress signal was oscillating or constant. When the signal diminishes exponentially, the apoptotic state disappears, and low suppression, as in the pre-cancerous malignant state, is limited by two critical points. This state allows the system to transition reversibly from an active to a pre-malignant state. If the stress is very high, it will cause the pre-malignant state to expand, moving the system to an irreversible cancerous state. Therefore, identifying the pre-malignant state may be essential for therapeutic interventions and cancer treatment [19].

Pathway analysis

The analysis continued by examining the interaction of the KEGG pathway hsa04218 (cellular senescence), yielding a pathway (Figure 2). The objective was to identify which proteins play the most significant roles in the KEGG pathway analysis, focusing on cellular senescence nodes, cell cycle, p53 signaling pathway, and mTOR signaling pathway. Results indicated that the proteins TP53, CDK2, and PTEN played the most prominent roles (Figure 3).

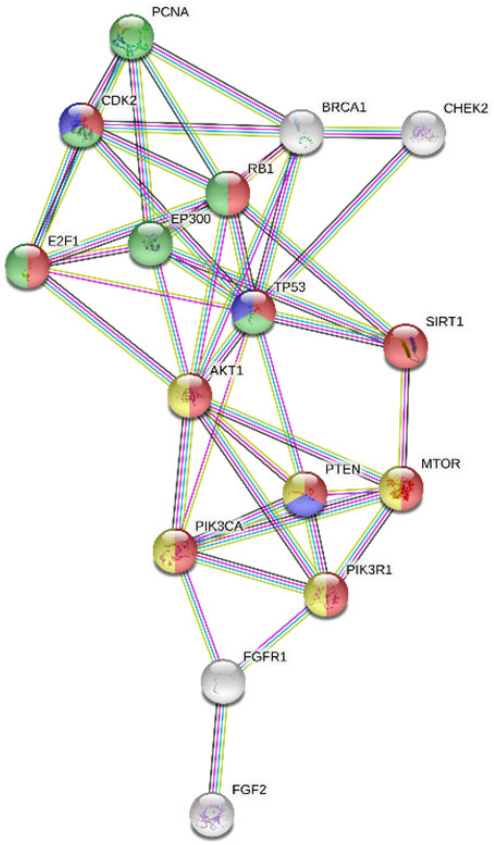

Figure 3. Protein protein interaction from analysis of cellular senescence nodes, cell cycle, p53 signaling pathway, and mTOR signaling pathway. The most important nodes are TP53, CDK2 and PTEN

Discussion

Premature senescence is a state of irreversible cellular arrest triggered by factors such as telomere shortening, oncogene activation, irradiation, DNA damage, and oxidative stress. It is characterized by flattened, enlarged cell morphology, aging-related beta-galactosidase (SA-β-gal) activity, and the secretion of inflammatory cytokines, growth factors, and matrix metalloproteinases as part of the senescence-associated secretory phenotype (SASP). Premature senescence is functionally related to various biological processes, including aging, tumor suppression, placental biology, embryo development, and wound healing [20].

During the cell cycle, multiple checkpoints ensure cellular health throughout the phases (G0, G1, S, G2, M) of the eukaryotic cell cycle. DNA damage activates these checkpoints to temporarily halt the cell cycle, allowing for DNA repair. If the damage cannot be repaired, it leads to permanent cell cycle arrest or cell death. DNA damage consists of single-strand breaks and double-strand breaks, which threaten chromosomal stability and are primary inducers of the DNA damage response [21]. The presence of aging cells exacerbates the development of a pro-inflammatory and immunosuppressive microenvironment, potentially triggering tumorigenesis. Aging of tumor cells and stroma is a common outcome of anti-cancer therapy; approaches that exploit the growth-inhibiting effects of aging while limiting adverse effects have significant clinical potential [21–23].

The in silico protein-protein interaction study on cardiac premature senescence, using four keywords, showed strong interactions between the proteins TP53, CDK2, and PTEN. The p53 protein is a transcription factor highly regulated by post-transcriptional modifications and plays a crucial role in cellular senescence, tumor suppression, and the aging of organisms. It regulates self-renewal, genome stability, and differentiation of normal and cancer stem cells. Additionally, the p53 and retinoblastoma (Rb)-p16 pathways modulate cell reprogramming efficiency to induce the generation of pluripotent stem cells (iPSCs) through cell senescence [24].

In premature senescence, the DNA damage response (DDR) mediated by the p53 protein is triggered by inducers such as reactive oxygen species (ROS) and ultraviolet (UV) light. In the DDR state, ATM kinase phosphorylates p53 at Chk2, leading to p53 accumulation by avoiding Mdm2-mediated proteasomal degradation and initiating the transcription of several p53 target genes. The first senescence-associated downstream target gene identified from p53 was *CDKN1A*, which encodes the p21Waf1/Cip1 cyclin-dependent kinase inhibitor (CKI). p21Waf1/Cip1 is a crucial mediator of p53-dependent cell cycle arrest following DNA damage. Studies have shown that p53 binds to and transactivates the p21Waf1/Cip1 promoter during cellular replication of aging human diploid fibroblasts. Low levels of p21Waf1/Cip1 protein prevent cellular senescence, defining it as a potent mediator of p53-regulated growth arrest and cellular senescence in response to multiple stresses and DNA damage [24].

Upon stimulation, p53 regulates the expression of many target genes involved in cell cycle arrest, DNA repair, senescence, and apoptosis. Some studies have shown that p53 plays a vital role in maintaining genome integrity through its role in the DNA damage response. Loss of p53 function promotes chromosomal instability, leading cells to enter senescence or apoptosis [25].

Premature senescence in cardiomyocytes has traditionally been recognized for inhibiting uncontrolled replication in proliferative cells. Recent evidence suggests that post-mitotic cells, such as Purkinje neurons, also develop senescence-like phenotypes. The mechanism underlying cardiomyocyte decline with aging remains undetermined due to a lack of specific senescence markers. In rats, studies have shown that senescence is associated with increased cardiomyocyte size, ROS production, telomere attrition, and high levels of p53 or p16Ink4a expression. Comparisons between young rats (4 months) and old rats (20-22 months) have shown increased left ventricular weight and cardiomyocyte volume, reduced cardiomyocyte numbers, and decreased ventricular function, indicating pathology [26–28].

Conclusion

From the GeneCards, Cytoscape, and StringDB data, the analysis of protein-protein interactions in cardiac premature senescence using the keywords: premature senescence, cardiovascular, cardiomyocytes, and p53, revealed that TP53, CDK2, and PTEN had the highest interaction scores.

Funding

None.

Declaration of interest

None.

Authors contributions

TS: Conceptualization, Methodology, Data Curation, Writing - Original Draft Preparation, Supervision; ARP: Investigation, Formal Analysis, Visualization, Writing - Review & Editing; NSH: Software, Validation, Resources, Writing - Review & Editing; F: Project Administration, Funding Acquisition, Writing - Review & Editing.

Received: August 31, 2022
Revised: June 4, 2024
Accepted: June 30, 2024
Published online: August 4, 2024

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