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Research Paper

Investigating the Mechanism of Arsenic-induced Ferroptosis in the Skin

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ABSTRACT

Background: Ferroptosis, an oxidative and iron-dependent cell death, is a new type of regulated cell death. There are few studies on the mechanisms of ferroptosis in the skin and related diseases. Arsenic is shown to induce ferroptosis cell death. This study aimed to decipher the relationship between arsenic exposure and ferroptosis cell death in the skin.

Methods: Arsenic-gene interactions were obtained. Then, skin-specific arsenic-gene interactions were screened. Ferroptosis-related genes were identified. Analysis of functional and biological interactions was performed to identify possible mechanisms.

Results: The arsenic-gene interactions and the ferroptosis-related genes showed an overlap of 59 genes. Functional enrichment, protein-protein interaction, and transcription factor (TF)/miRNA target gene interaction analyses were used to look into the mechanism of arsenic-induced ferroptosis in the skin. *ACTB*, *CTNNB1*, *HSPA8*, *SRC*, *RACK1*, *CD44*, and *SQSTM1* were identified as key proteins. Gene ontology analysis of these proteins indicated the mitochondrial morphology and functionality changes following arsenic-induced ferroptosis in the skin. *HIF1A* and *SP1* TFs regulate a large number of genes compared to other TFs. Ten miRNAs with high interaction with ferroptosis-associated genes were identified.

Conclusion: This work investigated the mechanism of arsenic-induced ferroptosis in the skin and identified key genes and regulators, and functional analysis highlighted the role of mitochondria in this skin exposure.

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1. Introduction

Arsenic is a natural metalloid that is widely found in food, soil, and water [1]. There is increasing evidence that arsenic is tightly linked to adverse health effects and significant risks for several diseases, including various cancers, such as skin and lung cancer, cutaneous diseases, cardiovascular disease, and type 2 diabetes [2]. In toxic doses, arsenic can lead to both acute and chronic adverse health effects on the human body. Skin and neurological changes are the earliest and most common manifestations of toxicity with arsenic since the skin is one of the most important defensive organs of the body and protects against endogenous and exogenous stresses. Skin manifestations due to arsenic include pigmentary changes, arsenic keratosis, and skin malignancies [3]. Arsenic mediates its toxicity by inducing oxidative stress, causing immune disruption, damaging DNA repair, and disrupting signaling pathways, which may decipher the complex disease manifestations found in arsenicosis [4]. Arsenic-induced skin disorders have been increasingly investigated, but the underlying mechanism is not completely elucidated. Cell death is an important event that plays a key role in maintaining skin homeostasis. In addition, dysregulation of cell death is increasingly defined as contributing to skin inflammation [5]. Excessive or poor apoptosis contributes to many disease processes, and several skin diseases can result from cell apoptosis both on a limited or large scale [6]. Ferroptosis is defined as an iron-dependent form of programmed cell death by the accumulation of lipid peroxidation that is different from apoptosis, necrosis, and autophagy [7]. Some heavy metals, such as arsenic are able to induce ferroptosis cell death [8]. Considerable research shows that ferroptosis plays a key role in diseases. In this regard, several studies have also confirmed the relationship between ferroptosis with various skin disorders [9]. Vast et al. reported that keratinocyte death via ferroptosis initiates skin inflammation after UVB exposure. Recently, it has been shown that ferroptosis activation owing to iron overload may be involved in the formation of skin lesions in psoriasis vulgaris [10]. Therefore, it can be assumed that ferroptosis is involved in skin disease caused by arsenic. Based on our knowledge, this study is the first comprehensive investigation of the underlying mechanisms involved in arsenic-induced ferroptosis in the skin, providing new insight for future ferroptosis research.

2. Materials and Methods

Data collection

Initially, arsenic-gene interactions were obtained through the CTD and STITCH databases [11, 12]. Subsequently, a skin tissue expression study of the arsenic-gene interactions was performed using the UP_TISSUE enrichment analysis available in the DAVID database to screen potential arsenic-gene relationships in the skin ($P<0.05$) [13]. The UP_TISSUE enrichment analysis exhibits over-expressed genes for various human tissues. In addition, ferroptosis-related genes were obtained through the FerrDb and GeneCards databases [14, 15]. Finally, the ferroptosis-related genes were intersected with the skin-specific arsenic-responsive genes to screen arsenic-induced ferroptosis-related genes in the skin.

Functional enrichment analyses of the ferroptosis-related genes

Gene ontology (GO) biological processes and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed by the DAVID functional annotation tool [13, 16]. A $P<0.05$ was selected for functional enrichment analyses.

Protein-protein interaction network construction

Protein-protein interactions (PPIs) of the ferroptosis-related genes were extracted from the STRING software, version 12 database using the default confidence score (≥0.400) [17] to construct arsenic-induced ferroptosis-related subnetwork in the skin by the Cytoscape software, version [18]. The plug-in network analyzer was used for topological analysis of the PPI network. GeneMANIA was employed to analyze the hub genes in the arsenic-induced ferroptosis-related subnetwork [19].

Identification of miRNA/TF-gene interactions

The experimentally validated miRNA-target interactions were retrieved from miRTarBase [20]. This database contains more than 360000 miRNA-target gene interactions, which are extracted by manual review of relevant literature. Also, the TF–target gene relationships were obtained from transcriptional regulatory relationships unraveled by sentence-based text mining (TRRUST) software, version 2 to predict the transcriptional regulatory interactions [21]. This database contains 8,444 TF-target regulatory interactions of 800 human transcription factors (TFs) that have been extracted from the PubMed articles.

3. Results

The purpose of this study was to investigate the mechanism of arsenic-induced ferroptosis in the skin. We retrieved the 5088 arsenic-gene interactions from CTD and STITCH databases. The 556 skin-specific arsenic-gene interactions were enriched by DAVID tissue expression analysis, considering the UP_TISSUE category using skin and keratinocyte significant terms. Based on the FerrDb and GeneCards databases, 2339 ferroptosis-related genes were obtained. The lists of skin-specific arsenic-gene interactions and the ferroptosis-related genes provided an overlap of 59 genes (Figure 1). The lists were used for functional enrichment analysis (Table 1 and Table 2). The gene ontology analysis results showed that the 59 genes were mainly enriched in negative regulation of the apoptotic process, positive regulation of the apoptotic process, negative regulation of transcription, DNA-templated, apoptotic process, and positive regulation of transcription, DNA-templated. The KEGG pathway enrichment showed fluid shear stress and atherosclerosis, necroptosis, *Salmonella* infection, Epstein-Barr virus infection, and lipids and atherosclerosis as the top significant pathways. PPI network analysis revealed arsenic-induced ferroptosis-related subnetwork in the skin with 56 nodes and 237 edges (Figure 2). As shown in Table 3, the top ten degree and betweenness centrality values showed an overlap of seven nodes as hub-bottleneck proteins. The hub-bottleneck proteins were queried in GeneMANIA to create a PPI network and function analysis (Figure 3). Hub-bottleneck proteins illustrated the PPI network with physical interactions of 62.13%, co-expression of 35.01%, co-localization of 1.84%, pathway of 0.55%, and prediction of 0.48%. Mitochondrial depolarization, the extrinsic component of the plasma membrane, cell-cell junction organization, regulation of mitochondrial membrane potential, regulation of membrane depolarization, membrane microdomain, and the extrinsic component of the membrane were identified as the main function of hub-bottleneck proteins (FDR <0.05). The transcription factors *HIF1A* and *SP1* were shown to target five and nine genes, respectively (Table 4). They regulated a large number of genes compared to other TFs. Additionally, ten miRNAs with high interaction with ferroptosis-associated genes were identified (Table 5).

4. Discussion

Based on the World Health Organization (WHO) reports, about 200 million people worldwide are exposed to arsenic in natural drinking water [22], which indicates that arsenic contamination is an important global public health problem. Chronic exposure to arsenic develops many potential skin diseases, including hyperkeratosis, hyperpigmentation, and various types of skin cancers [23]. Recent studies have shown that ferroptosis is closely associated with the occurrence of cutaneous diseases [9]. Therefore, in this study, to identify key genes and molecular mechanisms associated with arsenic-in-

Table 1. Gene ontology enrichment of arsenic-induced ferroptosis-related genes (the top 10 terms are presented)

Term	Genes	P
negative regulation of apoptotic process	*DDB1, PRDX2, NPM1, SRC, SPHK1, ID1, CTNNB1, MIF, PSEN1, CD44, HSP90B1*	0.000003
positive regulation of apoptotic process	*NR4A1, CDKN2A, SRC, ANKRD1, RACK1, CTNNB1, PSEN1, BID, SQSTM1*	0.0000055
negative regulation of transcription, DNA-templated	*PARP10, HSPA8, PARP1, CDKN2A, SRC, ID1, ANKRD1, CTNNB1, ENO1, BMAL1*	0.000057
apoptotic process	*DDB1, NR4A1, AHCYL1, PARP1, CDKN2A, IL1B, RACK1, PSEN1, BID, SQSTM1*	0.000082
regulation of neurogenesis	*ANXA2, IL1B, CTNNB1, BMAL1*	0.00023
positive regulation of transcription, DNA-templated	*NPM1, CDKN2A, SRC, IL1B, ANKRD1, CTNNB1, PSEN1, RUNX3, BMAL1, ACTB*	0.00027
protein folding in endoplasmic reticulum	*PDIA3, P4HB, HSP90B1*	0.00047
regulation of G1/S transition of mitotic cell cycle	*CDKN2A, PSME1, BID, ACTB*	0.00056
cellular senescence	*MAPK9, NPM1, CDKN2A, MIF*	0.00059
positive regulation of MAP kinase activity	*SRC, IL1B, MIF, PSEN1*	0.0016

Table 2. KEGG pathway enrichment of arsenic-induced ferroptosis-related genes (the top 10 terms are presented)

Term	Genes	P
Fluid shear stress and atherosclerosis	*MAPK9, SRC, IL1B, KEAP1, CTNNB1, SQSTM1, ACTB, HSP90B1*	0.0000087
Necroptosis	*MAPK9, PARP1, IL1B, BID, SQSTM1, FTL*	0.0016
Salmonella infection	*MAPK9, TUBA1A, ANXA2, IL1B, CTNNB1, ACTB, HSP90B1*	0.0021
Epstein-Barr virus infection	*PSMD8, PDIA3, MAPK9, BID, RUNX3, CD44*	0.0046
Lipid and atherosclerosis	*HSPA8, MAPK9, SRC, IL1B, BID, HSP90B1*	0.0059
Apoptosis	*MAPK9, TUBA1A, PARP1, BID, ACTB*	0.0062
Measles	*HSPA8, MAPK9, IL1B, RACK1, BID*	0.0067
Human cytomegalovirus infection	*PDIA3, CDKN2A, SRC, IL1B, CTNNB1, BID*	0.0072
Biosynthesis of amino acids	*CTH, IDH2, PGK1, ENO1*	0.0077
Shigellosis	*MAPK9, SRC, IL1B, SQSTM1, CD44, ACTB*	0.01

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duced ferroptosis in the skin, we first identified genes that overlapped between ferroptosis-related genes and skin arsenic-gene interactions. Then, the PPI network was constructed and seven hub bottlenecks were identified, which included *ACTB*, *CTNNB1*, *HSPA8*, and *SRC* (degree ≥20), *RACK1*, and *CD44* (degree=18), and *SQSTM1* (degree=17). Beta-actin (*ACTB*) is a cytoskeleton structural protein related to cell growth and migration and plays a main role in several human diseases. Actin filaments are one of the major elements of the cytoskeleton and contribute to TFRC pathway-mediated iron absorption [24]. It was also reported that arsenic induces a quick cell rounding and disruption of actin reorganization [25]. The *CTNNB1* hub gene plays an important role in cellular adhesion. Recently, it found that transgenic mice expressing an activated beta-catenin are susceptible to developing skin tumors [26]. Also, Chang et al. reported that chronic exposure to arsenic can induce neoplastic transformation, which may be associated with the β-catenin/c-Myc signaling pathway [27]. *HSPA8* is another hub gene identified in this work, which is involved in various cellular processes. Heat shock proteins (HSPs) are a group of stress proteins with protective effects that play a main regulatory role in several processes, including cellular homeostasis, cell proliferation and apoptosis, tumorigenesis and aging, and signal transduction. *HSP70* family members might increase cellular resistance to the ferroptosis event [28]. It can be concluded that *Hsp70* may be a graceful biomarker for arsenic exposure in humans. On the other hand, arsenic

Table 3. Hub-bottleneck proteins in arsenic-induced ferroptosis-related subnetwork

Gene Name	Degree	Betweenness Centrality	Description
ACTB	34	0.22202673	Actin beta (β-Actin)
CTNNB1	27	0.12486442	β-catenin
HSPA8	23	0.07768253	Heat shock protein family A (Hsp70) member 8
SRC	20	0.04256659	SRC proto-oncogene, non-receptor tyrosine kinase
RACK1	18	0.08361953	Receptor for activated C kinase 1
CD44	18	0.06946211	CD44 molecule
SQSTM1	17	0.09565501	Sequestosome 1

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Table 4. Key TF-gene interactions

Key TF	Description	P	List of Overlapped Genes
HIF1A	hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	5.55E-06	*BID, PGK1, MIF, NR4A1, ENO1*
SP1	Sp1 transcription factor	0.0000127	*NR4A1, MIF, CD44, SQSTM1, ME1, CDKN2A, SRC, PSEN1, DDB1*

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Table 5. Key miRNA-target gene interactions

miRNA	Target Gene
hsa-miR-615-3p	*CDKN2A, DHODH, ENO1, HSPA8, P4HB, PARP1, PRDX2, PSMD8, PTBP1, RPL15, RPL7, TPM3, TUBA1A*
hsa-miR-92a-3p	*ACTB, AHCYL1, BID, ENO1, HSP90B1, HSPA8, MAPK9, NPM1, P4HB, PTBP1, RPL15, RPS10, TPM3*
hsa-miR-26b-5p	*AGPAT3, BID, CTH, DHODH, ID1, IDH2, KEAP1, PSME1, TPM3*
hsa-miR-16-5p	*ACTB, AHCYL1, CD44, CDKN2A, FTL, HSP90B1, HSPA8, SQSTM1, TPM3, TUBA1A*
hsa-miR-1-3p	*ACTB, ANXA2, CD44, HSP90B1, PDIA3, PRDX2, PSME1, PTBP1, TPM3, TRIM26*
hsa-miR-484	*ACTB, ENO1, HSP90B1, P4HB, PARP1, PSMD8, RPS10, SPHK1, SQSTM1*
hsa-miR-20a-5p	*DHODH, HSPA8, KRT10, MAPK9, PGK1, RPS10, RUNX3, SQSTM1*
hsa-miR-17-5p	*DHODH, HSPA8, KRT10, MAPK9, PARP1, PTBP1, RPL7, RUNX3, SQSTM1*
hsa-miR-149-5p	*AGPAT3, BID, DDB1, HSPA8, IL1B, NPM1, PARP1, PSMD8, RPL15*
hsa-miR-124-3p	*ACTB, CDKN2A, CTNNB1, ID1, NR4A1, PARP1, PTBP1, SPHK1, TUBA1A*

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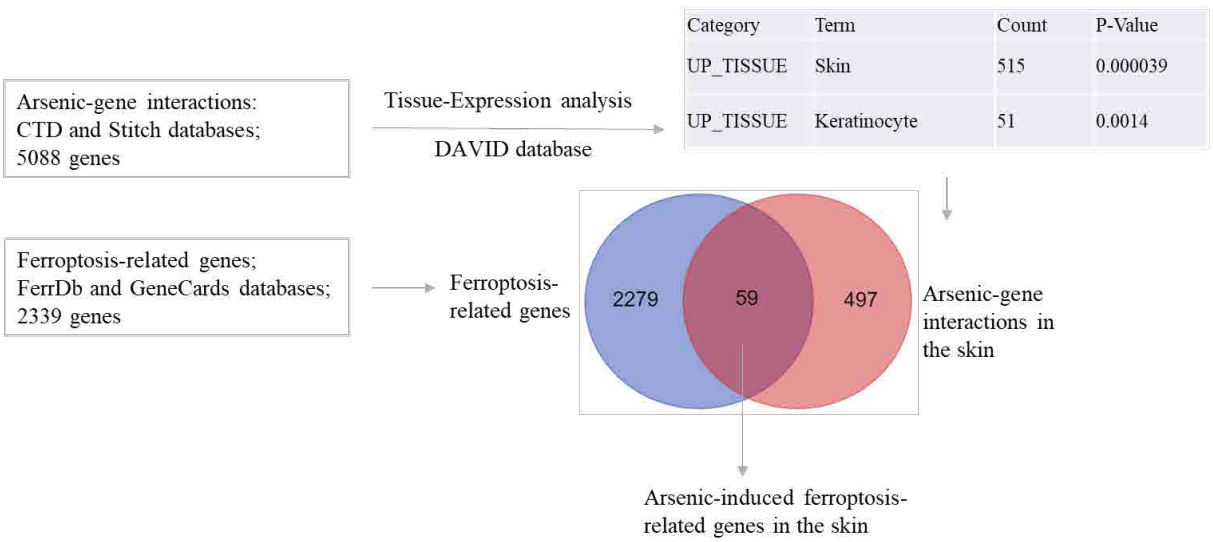

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Figure 1. A list of the 59 genes was obtained as arsenic-induced ferroptosis-related genes in the skin

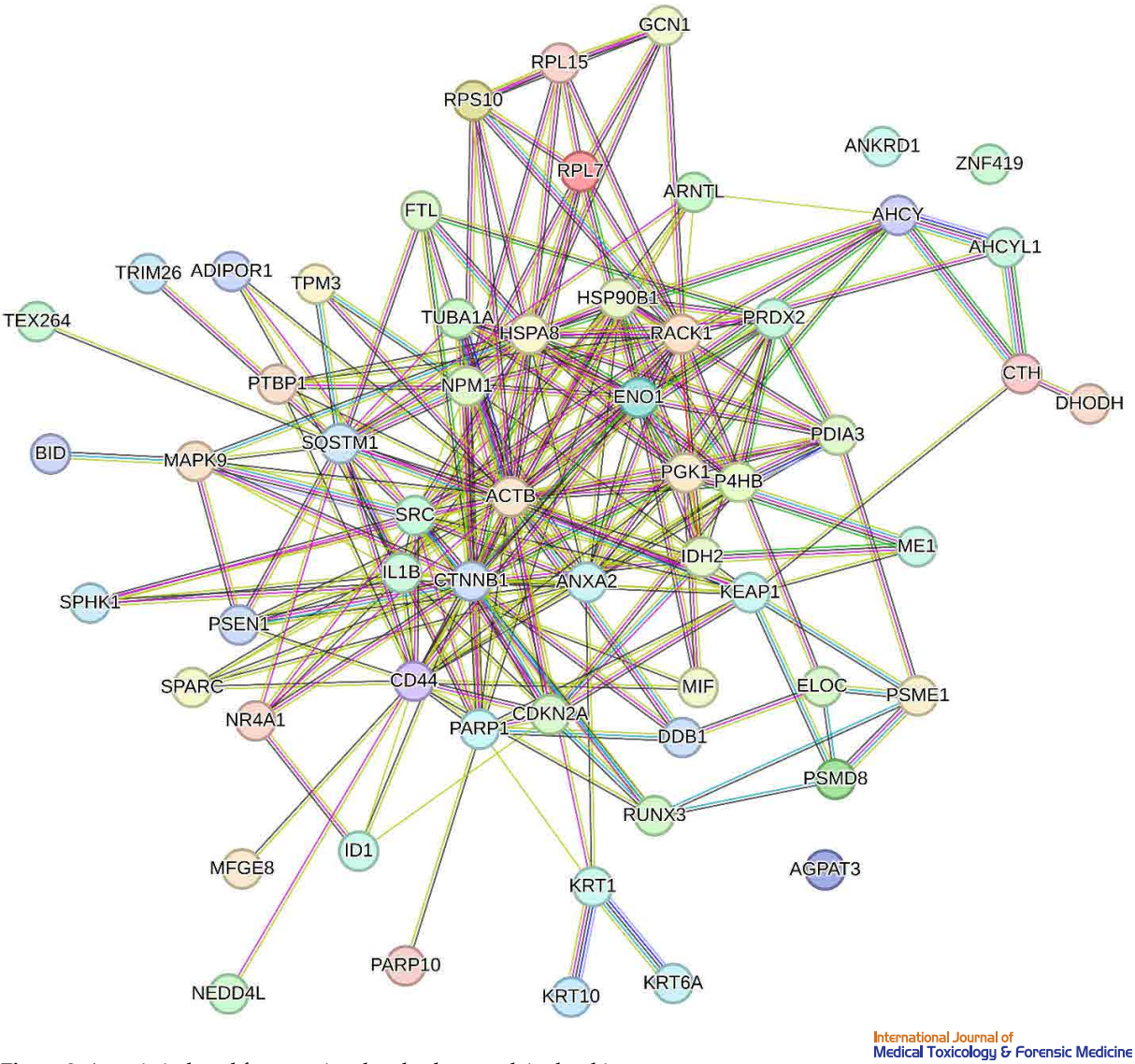

Figure 2. Arsenic-induced ferroptosis-related subnetwork in the skin

Fifty-six nodes with 237 interactions and 3 disconnected nodes are presented in the PPI network.

induces HSPs of different sizes. A signaling pathway triggered by heat stress, including arsenic, induces the activity of mitogen-activated protein kinases (MAP), extracellular regulated kinase (ERK), c-Jun-terminal kinase (JNK), and p38 [29]. The latest hub gene with a degree above 20 is proto-oncogene tyrosine-protein kinase Src (*SRC*). Recently, the first evidence of a molecular interaction between constitutive activation of tyrosine kinases and resistance to ferroptosis was provided by Cirotti et al. [30]. The PPI network and function analysis indicated the mitochondrial morphology and functionality changes as the main function of hub-bottleneck proteins following arsenic-induced ferroptosis in the skin. Increasing reports have suggested the possibility of multifaceted regulation of ferroptosis by mitochondria [31]. On the other hand, it has been demonstrated that arsenic can disrupt mitochondrial function [32]. Considering the enrichment of key genes in mitochondrial dysfunction, as well as the role of this organelle in the ferroptosis process, it is possible to take an important step by manipulating these genes in controlling diseases caused by ferroptosis in the skin. In the current study, two key transcription factors (*HIF1A* and *SP1*) were detected that target arsenic and ferroptosis-related genes in the skin. In a recent bioinformatics analysis, the ferroptosis-related gene *HIF1A* was one of the valid biomarkers for stomach adenocarcinoma [33]. In another study, *HIF1A* was identified as a ferroptosis-related hub gene that may affect the pathogenesis of chronic obstructive pulmonary disease via regulating ferroptosis [34]. *SP1* is involved in most cell processes, such as cell differentiation, cell growth, apoptosis, immune responses, and response to

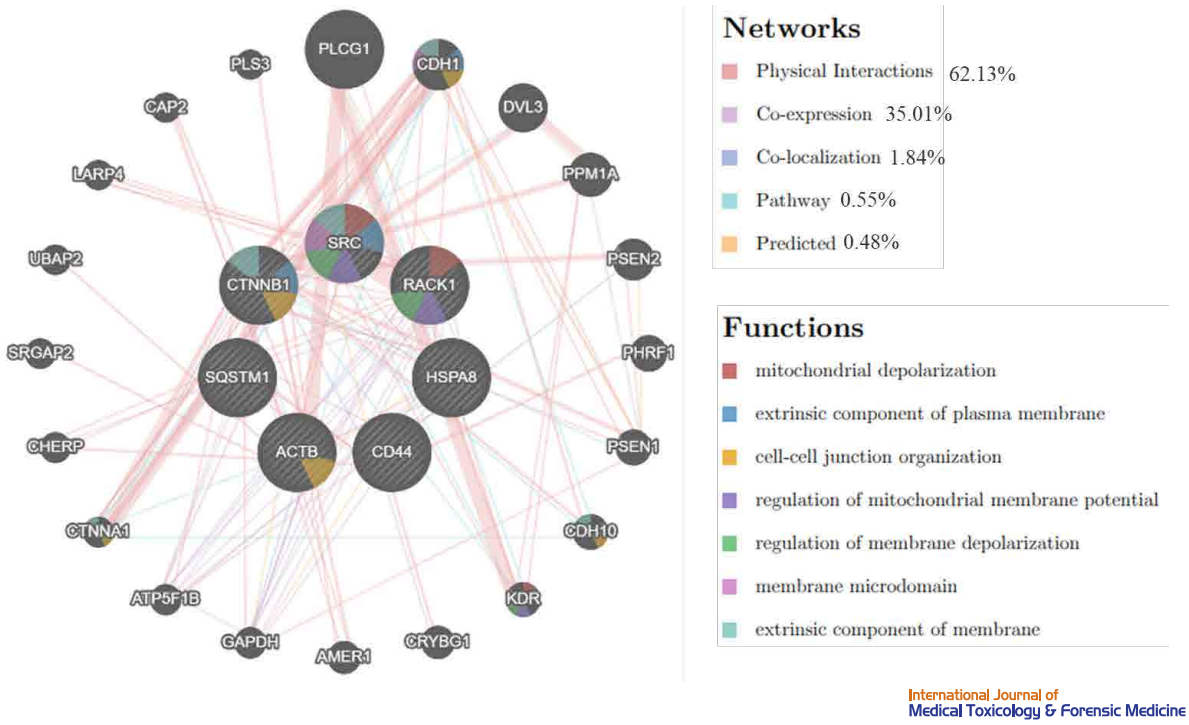

Figure 3. PPI network and functional analysis of hub-bottleneck proteins (inner circles) using GeneMANIA

DNA damage [35]. In our study, the top ten key miRNAs were represented. Several studies have revealed that microRNAs are involved in the occurrence of various diseases, such as cardiomyopathy, cancers, neurodegenerative diseases, etc. via stimulating or inhibiting ferroptosis [36]. In addition, it is known that the interactions between miRNAs and toxic metals might participate in the hazardous effects of these toxic elements in the body [37].

5. Conclusion

Our findings indicated seven hub genes as candidates for triggering arsenic-induced ferroptosis in the skin and identified key regulators of the process. Functional analysis of the key proteins highlighted the link between mitochondria and ferroptosis following arsenic exposure in the skin. Therefore, our study may help to understand the molecular mechanisms of ferroptosis stimulated by arsenic in the skin. However, the role of these ferroptosis-related genes needs to be investigated in future experiments, and more studies are required to decipher the relationship between mitochondria and ferroptosis in arsenic exposure.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Scientific investigation and writing–original draft: Mehdi Koushki and Nasrin Amiri-Dashatan; Conceptualization and supervision: Masoumeh Farahani; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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