Heliyon 9 (2023) e21052

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Interactive mechanism between connexin43 and Cd-induced autophagic flux blockage and gap junctional intercellular communication dysfunction in rat hepatocytes

Yuntian Duan [a,1], Yi Zhang [a,b,c,1], Tao Wang [a,b,c], Jian Sun [a,b,c], Waseem Ali [a,b,c], Yonggang Ma [a,b,c], Yan Yuan [a,b,c], Jianhong Gu [a,b,c], Jianchun Bian [a,b,c], Zongping Liu [a,b,c], Hui Zou [a,b,c,*]

[a] College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, China
[b] Joint International Research Laboratory of Agriculture and Agri−Product Safety of the Ministry of Education of China, Yangzhou 225009, China
[c] Jiangsu Co−Innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou 225009, China

ARTICLE INFO

Keywords:
Hepatocytes
Cadmium
Connexin 43
Autophagy
Rat

ABSTRACT

Cadmium (Cd) is a significant environmental contaminant known for its potential hepatotoxic effects. However, the precise mechanisms underlying Cd-induced hepatotoxicity have yet to be fully understood. Therefore, the purpose of this study was to investigate the dynamic role of connexin 43 (Cx43) in response to Cd exposure, particularly its impact on gap junctional intercellular communication (GJIC) and autophagy in hepatocytes. To establish an in vitro model of Cd-induced hepatocyte injury, the Buffalo rat liver 3A cell line (BRL3A) was utilized.In order to elucidate the mechanism by which Cx43 influences Cd-induced hepatocyte toxic injury, inhibitors of Cx43 (Dynasore) and P-Cx43 (Ro318220) were employed in the model. The findings revealed that inhibiting Cx43 and its phosphorylation further compromised GJIC function, exacerbating the impairment, while also intensifying the blockage of autophagic flux. To gain further insight into the role of Cx43, siRNA was utilized to knock down Cx43 expression, yielding similar results. The down-regulation of Cx43 expression was found to worsen the morphological damage induced by cadmium exposure, diminish the cell proliferation capacity of BRL3A cells, and exacerbate the disruption of GJIC and autophagic flow caused by Cd.These findings suggest that Cx43 may serve as a potential therapeutic target for the treatment of liver damage resulting from Cd exposure. By targeting Cx43, it may be possible to mitigate the adverse effects of Cd on hepatocytes.

1. Introduction

Cadmium is one of the most prevalent and harmful environmental metals, and becoming a major issue on a global scale due to industrial and agricultural production. The health of people and animals is seriously threatened by cadmium pollution. Therefore, cadmium can enter the body of organisms and accumulate to a high level in several organs [1]. Liver is the first vital organ that retains the cadmium, and store the many toxic chemicals. More than one-third of the body's total cadmium reserves are found in the liver,

* Corresponding author. College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, China.
E-mail address: zouhui@yzu.edu.cn (H. Zou).
[1] Joint first authors, these authors contributed equally to work.

https://doi.org/10.1016/j.heliyon.2023.e21052
Received 16 July 2023; Received in revised form 4 October 2023; Accepted 13 October 2023
Available online 14 October 2023

which is also extremely vulnerable to Cd-induced cytotoxicity [2]. Cadmium exposure can result in hepatocyte necrosis, swelling, and inflammatory cell infiltration, according to in vivo studies [3–5]. Studies conducted in vitro have demonstrated that cadmium can limit hepatocyte growth and proliferation, alter the morphology of hepatocytes, harm their ultrastructure, and cause hepatocyte mortality [6,7]. As a result of the ability of Cd-damaged hepatocytes to use GJIC to send apoptotic signals to nearby healthy hepatocytes, causing them to undergo apoptosis, the mechanism of Cd-induced liver damage involves GJIC significantly as well. Additionally, exposure to cadmium inhibits GJIC, and the inhibition of GJIC facilitates Cd-induced hepatocyte apoptosis [8,9]. These reports on the mechanism of cadmium toxicity lay a theoretical basis for resolving the effects of cadmium on liver health and pointed out the direction of research.

Physiological processes such as cell proliferation, differentiation, apoptosis, and death depend heavily on GJIC, a common method of communication between mammalian cells [10–12]. Gap junction channels are membrane channels composed of gap junction proteins, and this channel allows material exchange between cells. Different types of Cxs are engaged in many processes, including cell proliferation, adhesion, and intercellular signaling, either alone or as parts of gap junction complexes, depending on the type of ligand protein [13]. Cx43 has received the most research attention of all the connexins due to its expression in the majority of cell types. The ability of cadmium to increase Ca^{2+}-induced hepatotoxicity by inhibiting GJIC has been demonstrated. α-Lipoic Acid greatly relieve Cd-induced gap junction inhibition, considerable reduction of Cx43 mRNA, and gap junction channel breakdown [14]. It has been demonstrated that exposure to low concentrations of Cd increased the phosphorylation of the Cx43-Ser368 locus and mildly elevated GJIC and Cx43 levels in human prostate epithelial cell line RWPE-1 [15]. The production and localization of linker proteins and the opening and closing of gap junction channels are essential for the function of GJIC and are crucial for the survival of damaged cells. Cd-induced cellular autophagy is two-sided and has a protective effect on cells in a short period of time; the localization of connexins, which is closely related to GJIC, is affected by autophagy, which has been demonstrated in prior studies to be a significant pathway of connexins degradation [16].

Autophagy is one of the defense mechanisms that helps to promote cellular fitness and maintain cellular homeostasis. Autophagy can also remove intracellular pathogens and is therefore considered a survival mechanism for cell [17]. The occurrence and progression of many diseases are closely correlated with autophagy deficiency or dysfunction. Therefore controlling autophagy is crucial for effective disease management [18,19]. Autophagy activation and autophagic flux restoration have been shown to be essential defensive mechanisms against the toxic effects of lead or cadmium [20–23]. Numerous studies have now established that cadmium impacts autophagy, namely that low to moderate concentrations (e.g., 0.1–10 μM) of cadmium induce cellular apoptosis and activate cellular autophagy, but high concentrations (>50 μM) of cadmium result in cell necrosis [24]. Menghao Chen et al. [25] found that Cd induces dysregulation of the miR-33-AMPK axis, leading to BNIP3-dependent chicken spleen autophagy via AKT/mTOR and HSP70–NF-kB/JNK signaling pathways. Cadmium can also damage the rat renal cortex by inducing endoplasmic reticulum stress and causing autophagy [26]. Additionally, Cxs degradation via autophagy can regulate GJIC [27]. Cadmium suppresses GJIC activity and downregulates Cx43 expression, while GJIC has an antagonistic effect on Cd-induced autophagy and autophagic flux blockade in BRL3A cells, according to prior study [28]. Despite being a crucial part of GJIC, how Cx43 contributes to GJIC and autophagy is largely unknown.

Therefore, by developing an in vitro Cd-induced hepatocyte injury model, this study aims to further explore the effects of cadmium exposure on hepatocyte autophagy and GJIC function as well as the role of Cx43 in this process.

2. Materials and methods

2.1. Antibodies and reagents

Cd acetate hydrate (Cd, 229490), Lucifer Yellow CH dilithium salt (LY), Rhodamine B isothiocyanate-Dextran (RD), Ro318220 methanesulfonate salt (Ro), Dynasore hydrate (Dy), anti-LC3B, anti-p62/SQSTM1 were got from Sigma-Aldrich (St. Louis, MO, United States); Thermo Fisher Scientific (Waltham, MA, United States) supplied DMEM (Gibco, 12800-017) and FBS (Gibco, 10437-028); Cell Signaling Technology Inc. supplied anti-actin(4970S), anti-Atg7(2631S), anti-Beclin-1(3738S), anti-Cx43(Ser368), and antiPhospho-Cx43(Ser368) (3511S) (Danvers, MA, United States). Jackson ImmunoResearch provided all of the secondary antibodies (Philadelphia, PA, USA). RiboBio Corporation created the siRNA targeting Cx43 (Guangzhou, China). Beyotime Biotechnology provided the EdU-488 cell proliferation assay (Shanghai, China). All of the other resources were of analytical quality.

2.2. Cell culture and treatment

BRL 3A cells were taken from the Institute of Biochemistry and Cell Biology's cell collection (Shanghai, China). When the cell density approached approximately 90 %, the culture medium was discarded, and any remaining medium was rinsed off using PBS. Subsequently, the cells were subjected to trypsin digestion by adding a trypsin digestion solution. Once the cell gap expands and a small number of cells begin to separate, the trypsin digestion process needs to be stopped. The used trypsin digestion solution was then discarded. Following this, complete medium was added to terminate the digestion process, and the cell suspension was collected by gently blowing on the cells using a glass dropper. The collected cell suspension was centrifuged at 1300 rpm for 5 min to obtain a cell pellet. The cell pellet was carefully collected and supplemented with complete medium. The cells were then passaged into new flasks at a seeding ratio ranging from 1:2 to 1:4, with passages performed every 1–2 days to ensure optimal cell growth and proliferation.

2.3. Small Interfering RNA (siRNA) transfection

Cx43-specific siRNA was synthesized by the RiboBio (Guangzhou, China). The sequence of the siRNA that targets the mRNA for Cx43 is si-*r*-Gja1_ 003, 5′-GAACTACAGCGCAGAGCAA-3'. BRL3A cells were inserted into 6-well or 24-well plates to knock out Cx43. As per the guidelines provided by the manufacturer, BRL3A cells were transfected with 20 nmol/L Cx43 siRNA or a negative control siRNA under conditions of 40 % confluence for 24 h using transfection reagents (Polyplus Transfection, Illkirch, France). Then, collect the cells and analyze the knockout efficiency with a immunoblots or conduct other treatments according to the experimental requirements.

2.4. Analysis of cell morphology

BRL3A cells were inoculated in a 24-well plate and cultured for 24 h in a fresh medium free of penicillin and streptomycin with the transfection reagent and 20 nmol/L Cx43 siRNA or negative control siRNA at 40 % confluence. Cells were cultured to approximately 90 % fusion and treated with 5 μmol/L Cd for 6 h afterwards, cell morphology was observed using a Leica DM IRB inverted microscope.

2.5. Scrape loading/dye transfer assay

Following treatment, GJIC was calculated using the scrape-loading/dye-transfer method (SL/DT). Briefly, three times BRL3A cells were washed in the PBS, scraped, and then treated at 37 °C for 3 min with 0.5 mg/ml LY and 2.5 mg/ml RD before being fixed in 4 % paraformaldehyde. Finally, a fluorescence microscope (Leica DMI3000 B, Solms, Germany) was used to observe the diffusion distance of the dye in the cells on both sides of the scratch. Data were calculated for the mean and standard deviation ($n = 6$).

2.6. Immunoblotting analysis

After the completion of treatment, the cells were lysed and extracted by ultrasonic radiation immunoprecipitation analysis (RIPA), buffered and washed three times with PBS. BCA protein assay kit (Beyotime, China) was used to detect total cell protein concentration. SDS-PAGE is used to separate the same amount of total protein (20 μg) and transfer it to the polyvinylidene fluoride (PVDF) membrane. PVDM membrane was Incubate in 5 % skimmed milk for 2 h at 37 ^{O}C, and then soak the membrane in the primary antibody at 4 °C for overnight incubation. After 12 h, the diluted secondary antibody was incubated at room temperature for 2 h. Finally, the enhanced chemiluminescence (ECL) test kit was used to observe the protein band, and the ImageJ software was used to analyze the grey value of the protein band.

2.7. Infection and analysis of the StubRFP-SensGFP-LC3 Lentivirus

The GeneChem Corporation provided the StubRFP-SensGFP-LC3 (RFP, red fluorescent protein; GFP, green fluorescent protein) lentivirus (Shanghai, China). According to the manufacturer's instructions, BRL3A cells were seeded in a 24-well plate, grown to about 20 % confluence, and then exposed for 48 h to StubRFP-SensGFP-LC3 Lentivirus. Then, to create a stable cell line, choose positive cells using 4ug/mL puromycin (Solarbio). At 90 % confluence, replace the complete medium with serum-free medium and then handle according to the experimental requirements. Finally, the fluorescence image of the LC3 spot was observed with a fluorescence microscope (TCSSP8STED, Leica). Autophagic lysosomes are indicated by red puncta while autophagosomes are indicated by yellow puncta.

2.8. Analysis of cell proliferation

The EdU 488 kit was employed to evaluate cells' capacity to proliferate. Under a state of 40 % confluence, In fresh media devoid of penicillin and streptomycin, the cells were cultured with transfection reagents (Polyplus Transfection, Illkirch, France) and 20 nmol/L Cx43 siRNA or negative control siRNA, followed by a 12 h treatment with 5 μmol/L Cd. Cells were incubated in EdU working solution for 2 h before being treated with the 4 % paraformaldehyde, 0.3 % TritonX-100, ClickAdditivition, and Hoechst 33342 per the manufacturer's instructions. Finally, images were taken using a Leica DM IRB inverted microscope (DMI3000B, Leica, Wetzlar, Germany). Proliferative cells were recognized as those that were positive for EdU.

2.9. Statistical analysis

All the numerical data were analyzed using one-way analysis of variance (ANOVA) and presented as the mean ± SD using the (GraphPad Software, United States). $P < 0.05$ indicated a significant difference, and $P < 0.01$ indicated a highly significant difference.

3. Results

3.1. Inhibition of Cx43 expression or suppression of Cx43 phosphorylation exacerbates the inhibitory effect of Cd on GJIC function

The Cx43 inhibitor Dy and the p-Cx43 Ro were employed to modulate both Cx43 and p-Cx43 to examine the involvement of Cx43 in

Cd-induced cytotoxicity. Compared to the effect shown in the Cd group, the combination treatment of Dy and Cd hindered GJIC function, and the combined treatment of p-Cx43 and Cd obtained the same results (Fig. 1A-B) . Immuno blot analysis demonstrated that Cx43 levels were significantly reduced ($P < 0.05$) in the Dy–Cd combination treatment group compared to the Cd treatment group, but p-Cx43 (Ser368) levels were highly significantly higher ($P < 0.01$). p-Cx43 levels in the Ro-Cd combination treatment group were substantially lower ($P < 0.05$) than the comparable levels in the Cd treatment group, and Cx43 levels were also highly significantly reduced ($P < 0.01$) (Fig. 1C–E). The above results indicate that inhibition of Cx43 expression or suppression of p-Cx43 levels exacerbated the inhibitory effect of Cd on GJIC function in BRL3A cells.

3.2. Inhibition of Cx43 expression or suppression of Cx43 phosphorylation exacerbates Cd-induced blockade of autophagic flux

Our previous study showed that 6 h of Cd treatment resulted in the highest levels of autophagy [9]. So, the major time point for our investigation into the connection between autophagy and Cx43 was 6 h of pretreatment. After 6 h of Cd treatment, we used stubRFP and sensGFP markers to track LC3 and autophagy-lysosome fusion to ascertain the relationship between Cx43 and autophagy during Cd exposure. According to the findings, the number of yellow LC3 puncta and accumulation of patches around the nucleus were increased in Dy–Cd co-treated group compared to Cd alone, while the combined treatment of Ro and Cd also had the same effect (Fig. 2A). Immunoblots analysis revealed that the expression levels of Beclin-1, LC3II, and P62 were significantly higher after receiving a combination of Dy and Cd treatment ($P < 0.05$) compared to the Cd treatment group. In contrast, Atg7 expression remained constant, suggesting that blocking Cx43 function could further halt the autophagic flux in Cd-induced cytotoxicity. In contrast to the Cd alone, the combined treatment of Ro and Cd highly increased the expression levels of LC3II and Beclin-1 ($P < 0.05$, $P < 0.01$) and decreased the expression level of Atg7 ($P < 0.01$), while P62 did not change significantly (Fig. 2B–F). The above results suggest that inhibition of Cx43 and p-Cx43 expression exacerbated the Cd-induced blockage of autophagic flux in BRL3A cells.

3.3. Cx43 knockdown aggravated Cd-induced BRL3A cell damage

In order to further explore the mechanism of Cx43 in Cd-induced cell damage, the effect of Cx43 siRNA or negative control siRNA alone or in combination with 5 μmol/L Cd on the proliferation of BRL3A cells was detected by EdU staining. The findings revealed that the NC + Cd group's number of EdU-positive cells was considerably lower than that of the negative control group (NC). Compared with the NC + Cd group, the number of EdU-positive cells further decreased after si-Cx43 combined with Cd treatment (Fig. 3A). Next, we verified the protective effect of Cx43 on Cd-exposed BRL3A cells by changes in cell morphology. Results As seen in Fig. 3B, the NC + Cd

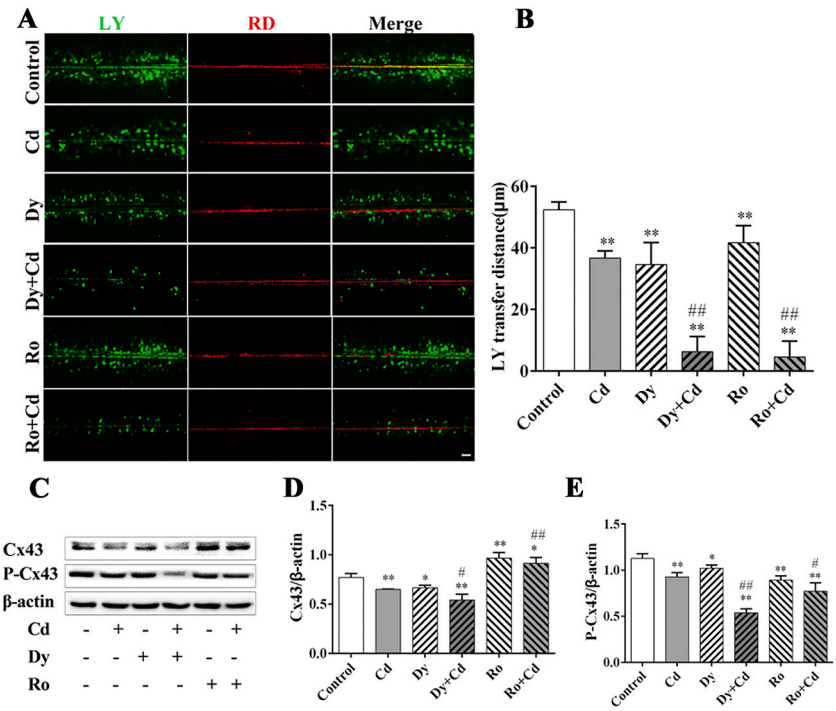

Fig. 1. Inhibition of Cx43 expression or suppression of Cx43 phosphorylation further exacerbates the inhibitory effect of Cd on GJIC function. (A–B) GJIC was measured using the SL/DT method with a scale bar of 50 μm after a combined treatment of 5 μmol/L Cd with 10 μmol/L Dy or 200 nmol/L Ro for 6 h. (C–E) The effects of Dy and Ro on Cx43 and p-Cx43 expression levels were analyzed by immunoblotting. Compared with the control group, $^{*}P < 0.05$, $^{**}P < 0.01$; with the Cd treatment group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

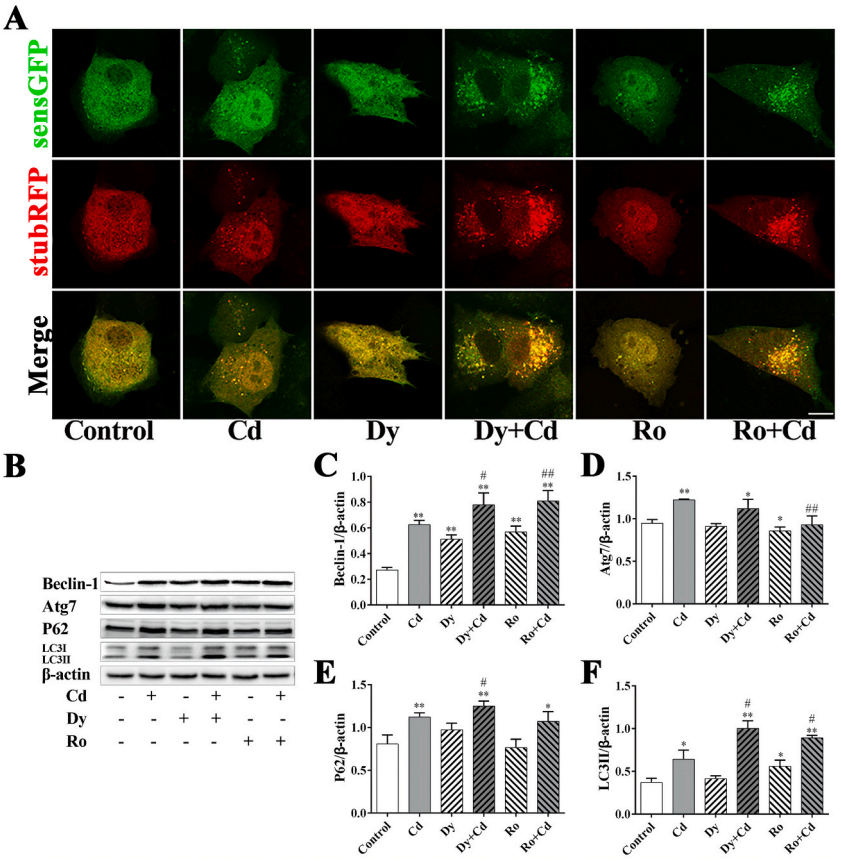

Fig. 2. Inhibition of Cx43 expression or suppression of Cx43 phosphorylation exacerbates Cd-induced blockade of autophagic flux. (A) BRL 3A cells were transfected with the stubRFP-sensGFP-LC3 lentivirus and subsequently treated with Cd or inhibitors of Cx43/p-Cx43. LC3 puncta were visualized by confocal microscopy. Scale bar = 20 μm. (B–F) By using immunoblotting, the effects of Dy and Ro on the expression levels of proteins related to autophagy were examined. Compared with the control group, $^{*}P < 0.05$, $^{**}P < 0.01$; with the Cd alone, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

treatment group's cell morphology underwent significant change, with shrinkage and distortion of the cells, enlargement of the gap, and suspension of dead cells. The morphological degradation of the cells was exacerbated, and the number of dead cells increased after Cd and si-Cx43 treatment was combined. These findings imply that when cadmium harm BRL3A cells, Cx43 acts as a preventative measure.

3.4. Knockdown of Cx43 exacerbates Cd-induced GJIC dysfunction in BRL3A cells

We employed Cx43siRNA in cell models to further confirm the crucial part that Cx43 plays in Cd-induced GJIC malfunction in BRL3A cells. Compared with the negative control (NC) group, LY diffusion distance in the NC + Cd group was significantly decreased ($P < 0.01$) (Fig. 4A,C). Compared with the NC + Cd group, LY diffusion distance was significantly decreased after combined treatment with Cd and si-Cx43 ($P < 0.01$). Immunoblots analysis showed that the expression level of Cx43 in the si-Cx43 group was significantly decreased compared with that in the NC group ($P < 0.05$); Compared with the NC + Cd group, the expression level of Cx43 was significantly decreased after combined treatment with Cd and si-Cx43 ($P < 0.05$) (Fig. 4B,D). These findings indicated that Cx43 reduced the malfunctioning of the GJIC brought on by Cd in BRL3A cells.

3.5. Knockdown of Cx43 exacerbates Cd-induced autophagic flux blockade in BRL3A cells

To more clearly verify how Cx43 is crucial for the Cd-induced autophagy retardation of BRL3A cells, we followed LC3 with stubRFP and sensGFP markers to monitor autophagosomal lysosomal fusion. According to the findings, there were more yellow LC3 puncta in the Cx43 knockdown group than in the NC group. Following combination treatment with Cd and si-Cx43, the accumulation of yellow LC3 puncta was increased in comparison to the NC + Cd group. (Fig. 5A). Immunoblotting analysis showed that compared with the NC + Cd group, the expression levels of LC3II and P62 were significantly increased after combined treatment with Cd and si-Cx43 ($P < 0.05$) (Fig. 5B–D). These phenomena are consistent with previous experimental results, confirming that Cx43 plays a protective role in Cd-induced autophagic flux arrest in BRL3A cells.

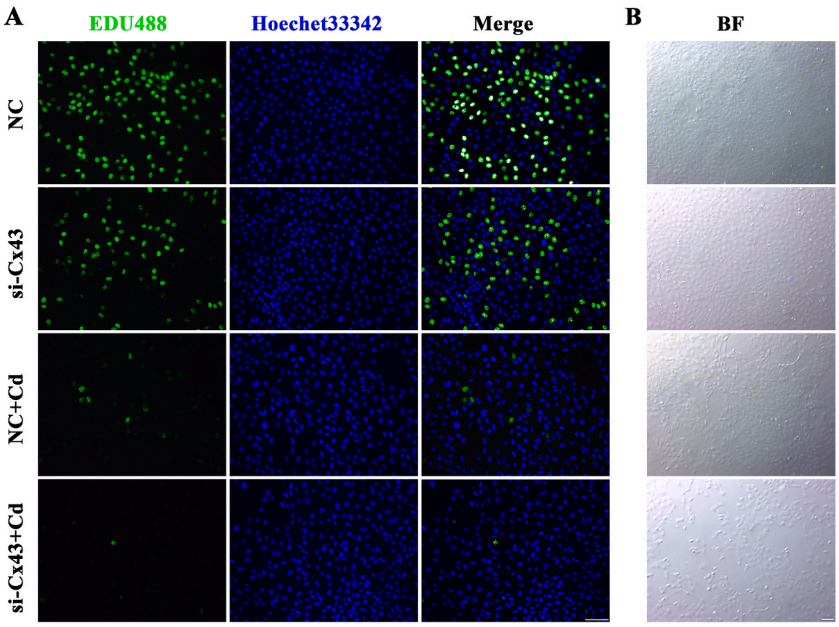

Fig. 3. Cx43 knockdown aggravated Cd-induced BRL3A cell damage. Based on the treatment with Cx43 siRNA or NC siRNA for 24 h. (A) Cell proliferation was identified by EdU staining after either a 5 μmol/L Cd treatment or a control treatment for 6 h. Scale bar = 100 μm. (B) The impacts of Dy and Ro on cell damage brought on by Cd therapy are visible in bright-field pictures. Scale bar = 100 μm.

4. Discussion

Cadmium is a highly toxic heavy metal that can enter the human body via various pathways to accumulate in the body and is difficult to excrete once it has entered the body. Cadmium toxicity is capable of causing damage to various organs in the body, and liver damage is an important feature of Cd-induced toxicity [29,30] and can potentially cause liver cancer [31]. Cadmium causes parenchymal injury and inflammatory cell infiltration and increases the activities of plasma alanine, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and alkaline phosphatase in the liver [32]. The damaging effects of cadmium have been shown at redox, metal status and transaminase levels [33]. It has been found that Cx43 siRNA attenuates Cd-induced apoptosis and inhibits the proliferation of HK-2 cells [34].To investigate the intrinsic relationship between Cx43 and heavy metal toxicity, we designed experiments to investigate the mechanism of Cx43 action during Cd-induced hepatotoxicity. Primary hepatocytes are rather difficult to prepare and genetically manipulate, and the BRL 3A cell line is one of the most commonly used cell lines in studies of hepatotoxicity. Therefore, we selected BRL 3A cells to construct a model of Cd-induced hepatocyte injury. Consistent with previous observations, the cadmium dose used in this study was 5 μmol/L [9,35]. The morphological changes and proliferative ability of BRL3A cells can reflect the toxic effects of the toxin on animal bodies and organs. Therefore, in this study, BRL3A cells were treated with Cx43 siRNA or negative control siRNA alone or in combination with 5 μmol/L Cd, and morphological changes and proliferative capacity changes of BRL3A cells were observed by EdU staining and microscopy. These results demonstrated that BRL3A cells exposed to cadmium for 6 h exhibited damage and decreased proliferative capacity; cells in the si-Cx43 + Cd group exhibited more severe changes in morphology compared to those in the NC + Cd group, the cells became round and detached, necrotic cells increased and proliferative capacity also decreased further, which was consistent with the findings of others [28]. Based on the above results, the model of Cd-induced hepatocyte injury was successfully established, and the knockdown of Cx43 exacerbated cell injury induced by cadmium, demonstrating a protective role for Cx43 in Cd-induced cell injury.

GJIC is key to maintaining the liver's internal balance. Stimulation of hepatocytes by external harmful substances impairs the structure and function of the hepatocyte gap junction channels, leading to changes in the expression of associated gap junction proteins, which in turn leads to a series of GJIC-mediated cascades leading to liver cell damage. Recent studies have demonstrated that cadmium can inhibit gap junction communication, negatively regulate connexin expression, block information exchange, and result in apoptosis, autophagy, and even carcinogenesis in cells [36]. The mechanism of regulation of GJIC is complex and associated with various factors such as connexin localization, expression, and phosphorylation levels. In previous laboratory studies, we have found that changes in GJIC during cell death do not correspond to the expression of the connexin Cx43, and the mechanism of Cx43 in Cd-induced GJIC dysfunction is still unclear. Cx43 is the most prevalent and critical GJ protein and plays an important role in many tissues. In different organs or at different times, the GJIC may play different roles. For example, blocking the GJ channel was found to have hepatoprotective effects in toxic liver injury [37,38] and ischemic injury to the brain [39] and heart [40] was shown to spread to uninfected cells via Cx43-mediated cell-to-cell communication. In bone tissue, CX43-mediated cell-to-cell communication has also proven to be an important way to regulate bone balance. Therefore, it is important to clarify the role of CX43 in cadmium-induced liver

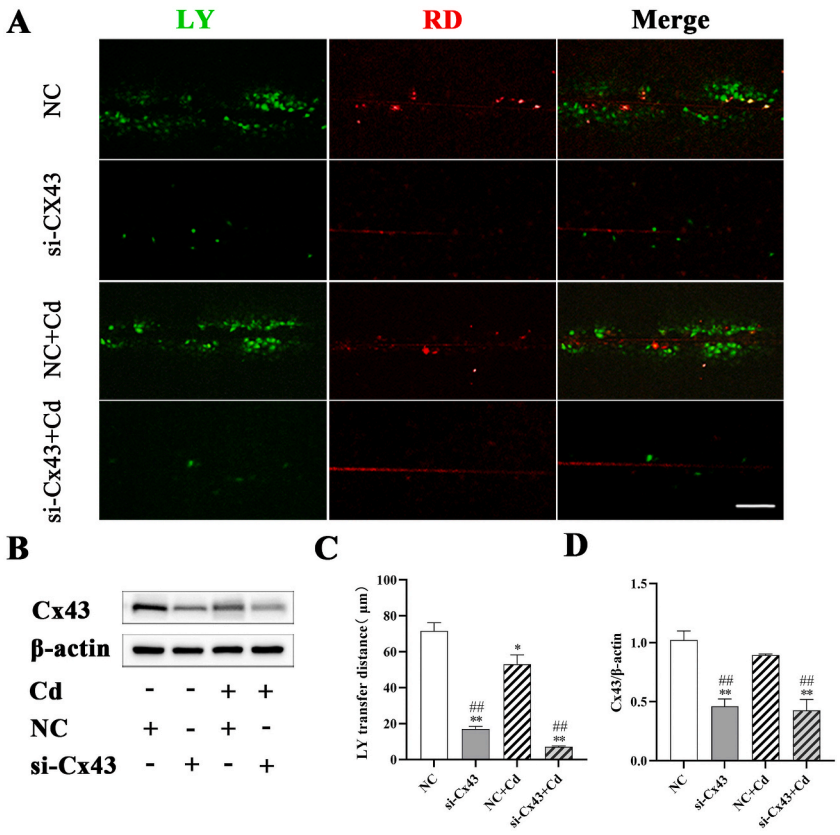

Fig. 4. Knockdown of Cx43 exacerbates Cd-induced GJIC dysfunction in BRL3A cells. Based on the treatment with Cx43 siRNA or NC siRNA for 24 h. Cells were treated with or without 5 μmol/L Cd for 6 h, (A, C) GJIC was measured by the SL/DT method, Scale bar = 100 μm. (B,D) The effects of Cx43 siRNA on Cx43 expression levels were analyzed by immunoblotting. Compared with the NC group, *P < 0.05, **P < 0.01; compared with the NC + Cd group, #P < 0.05, ##P < 0.01.

damage.

In contrast to other gap junction proteins, Cx43 turnover occurs at an extremely fast rate, and the full turnover cycle of Cx43 is estimated to take only 1.5–3 h. The normal function of GJIC is tightly linked to the ongoing synthesis and degradation of connexins. As for the choice of Dy and Ro concentrations, we conducted a preliminary experiment. The effect of Dy on LY diffusion distance was observed in cells treated with different concentrations for 6 h. Compared with the control group and 2.5 μmol/L Dy, the diffusion distance of 10 μmol/L Dy LY was significantly reduced. The effect of Ro318220 on LY diffusion distance was observed after the cells were treated with different concentrations for 6 h. The LY diffusion distance of 200 nmol/L Ro, as a Cx43 phosphorylation inhibitor, was significantly decreased compared with the control group and 50 nmol/L Ro. These results provided evidence for subsequent tests. This study initially used Dy, an inhibitor of Cx43, to inhibit Cx43 expression from limiting GJIC function and observed changes in GJIC function and expression levels of Cx43 upon Cd-induced injury of BRL3A cells. The results showed that GIJC function was significantly decreased, and the Cx43 expression level was significantly decreased in Dy + Cd group compared with Cd treatment group. This suggests that Cx43 plays a protective role in Cd-induced GJIC dysfunction in BRL 3A cells. Subsequently, to further validate the above conclusion, we knocked down Cx43 using Cx43 siRNA and came to the same result.

Autophagy is a process of autodigestion and degradation of cells that can provide materials and energy to synthesize other proteins [41]; researchers regard it as an important survival mechanism of cells [17]. Autophagy is a possible therapeutic target for numerous disorders, including cancer, autoimmune diseases, neurodegenerative diseases, and metabolic syndrome diseases [19]. Autophagic flux refers to the rate at which autophagy degrades cargo [42]. Blocking autophagic flux triggers autophagy dysfunction and ultimately cell death [43].Modulation of autophagic flux is a key therapeutic target for a number of diseases where autophagy blockage is implicated, including a number of malignancies, cardiovascular conditions, and liver disorders [44,45]. The important thing is, autophagic flux has been shown to be a major mechanism of heavy metal toxicity [46,47]. Reduced autophagy blockage may lessen the cell damage caused by heavy metals. The autophagy-related protein LC3 has been identified as a characteristic of mammalian autophagy in recent investigations, and the ratio of LC3II to LC3I is the most widely used index to evaluate autophagy. P62, a ubiquitin system adapter protein, is crucial for associating ubiquitinated proteins and for the fusion of autophagosomes and lysosomes [48]. P62 reflects the total level of degradation caused by autophagy, while LC3 measures the overall level of autophagy. P62 and LC3 levels are increased when late autophagy is inhibited [49,50]. Cadmium increased the expression of Atg family proteins and autophagy SNARE

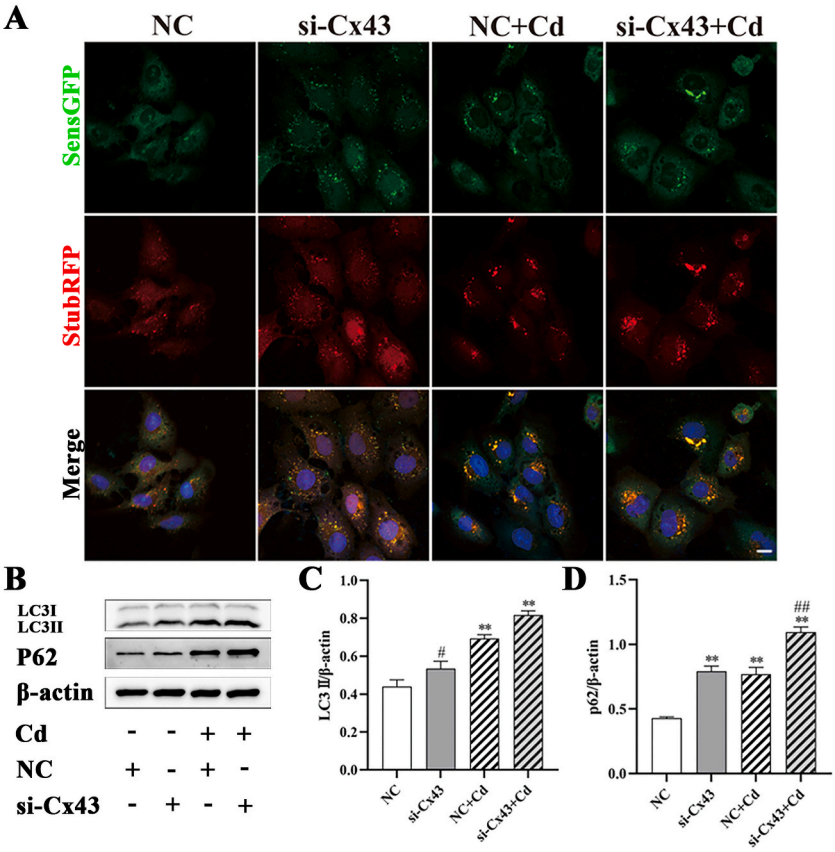

Fig. 5. Based on a 24 h treatment with either NC siRNA or Cx43 siRNA. (A) BRL 3A cells were transfected with the stubRFP-sensGFP-LC3 lentivirus and subsequently treated with or without 5 μmol/L Cd. Confocal microscopy was used to observe LC3 puncta. Scale bar = 20 μm. (B–D) Immunoblotting was used to examine how Cx43 siRNA affected the expression levels of proteins related to autophagy. Compared with NC group, $^{**}P < 0.01$; compared with the NC + Cd group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

proteins (STX8, STX17) in prostate epithelial cells and protected cells from cadmium toxicity, indicating that the autophagy machinery is positive [51]. Cadmium exposure also induced structural changes in PC12 cells and promoted autophagy, and Beclin-1 may play an important regulatory role in Cd-induced autophagic changes in PC12 cells.

Along with immunoblotting analysis, another widely used technique to track autophagic flux is stubRFP-sensGFP-LC3 fluorescent spot analysis [51]. Previous studies have shown that autophagy is the main pathway of Cxs degradation and is involved in connexin localization [16], which has a close relationship with GJIC. As an important component of GJIC, it remains unclear how Cx43 impacts autophagy and how they interact. In this study, we investigated the effects of Cx43 inhibitor Dy and p-Cx43 inhibitor Ro on autophagy by treating BRL3A cells with 5 μmol/L Cd for 6 h to achieve the highest level of autophagy. The results showed that cadmium increased orange fluorescence accumulation points compared to the control group, indicating that cadmium caused autophagic flux arrest. In contrast, cadmium combined with Cx43 inhibitor Dy further increased LC3 orange fluorescence accumulation points, and the expression levels of autophagy-related proteins Beclin-1, P62 and LC3II were significantly increased, indicating that the inhibition of Cx43 exacerbated Cd-induced autophagic flux arrest in BRL3A cells. We used Cx43 siRNA in a cell model to further validate this result. Knockdown of Cx43 postconditioning cells showed that after co-treatment with si-Cx43 and Cd, orange sunshine accumulation points were further increased, and the expression levels of P62 and LC3II were significantly increased compared with the NC + Cd group. These phenomena are consistent with previous experimental results. In summary, the reduction of Cx43 exacerbated Cd-induced autophagic flux block in BRL3A cells, possibly due to the ability of Cx43 reduction to inhibit GJIC function, consistent with previous studies that autophagy showed negative regulation with GJIC.

Connexins and their channels have been demonstrated to underlie a range of liver diseases, such as drug-induced acute liver failure, hepatitis, nonalcoholic fatty liver disease, cirrhosis, and hepatocellular carcinoma [52,53]. It is important to note that connexins, like Cx43 and Cx32, have been shown to be crucial in the disease development process in cancer research. They can also be used as biomarkers of metastasis and survival prognosis and are crucial in metastasis and chemoresistance. Therefore, various connexins may have varying effects on the onset of disease and therapy resistance. However, connexin action in Cd-induced liver damage has not been fully explained. In the present study, we demonstrate a link between Cx43 and Cd-induced liver injury, whereas what other connexins play a role in this process remains to be investigated further. Except for Cx43 and Cx32, other connexins have been less studied. In the

future, we will do further research on the mechanism of connexin in Cd-induced liver injury, which may lead to the discovery of new biomarkers and targets for the therapeutic development of liver diseases.

5. Conclusion

In conclusion, inhibition of Cx43 exacerbates Cd-induced BRL 3A cell injury and leads to autophagic flux blockade and GJIC dysfunction. Cx43 plays a protective role during Cd-induced GJIC dysfunction and autophagic flux block in BRL 3A cells. Therefore, we suggest that Cx43 is an important target for regulating autophagic flux and GJIC to mitigate cadmium hepatotoxicity. This study provides new insights into the investigation of cadmium hepatotoxicity.

Funding

This work was supported by the National Natural Science Foundation of China (Nos. 31702305, 31872533, 31902329, and 32072933), the Undergraduate Research & Practice Innovation Program of Jiangsu Province (202211117003Z), and a Project Funded by Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Data availability

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Yuntian Duan: Conceptualization, Writing – original draft. **Yi Zhang:** Formal analysis, Writing – original draft. **Tao Wang:** Data curation, Formal analysis. **Jian Sun:** Data curation, Formal analysis. **Waseem Ali:** Data curation, Formal analysis. **Yonggang Ma:** Data curation, Formal analysis. **Yan Yuan:** Data curation, Formal analysis. **Jianhong Gu:** Data curation, Formal analysis. **Jianchun Bian:** Validation. **Zongping Liu:** Validation. **Hui Zou:** Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21052.

References

[1] C.N. Amadi, S.J. Offor, C. Frazzoli, O.E. Orisakwe, Natural antidotes and management of metal toxicity, Environ. Sci. Pollut. Control Ser. 26 (2019) 18032–18052, https://doi.org/10.1007/s11356-019-05104-2.
[2] C.E. Imafidon, O.S. Olukiran, D.J. Ogundipe, A.O. Eluwole, I.A. Adekunle, G.O. Oke, Acetonic extract of Vernonia amygdalina (Del.) attenuates Cd-induced liver injury: potential application in adjuvant heavy metal therapy, Toxicol Rep 5 (2018) 324–332, https://doi.org/10.1016/j.toxrep.2018.02.009.
[3] T. Li, H. Yu, Y. Song, R. Zhang, M. Ge, Protective effects of Ganoderma triterpenoids on cadmium-induced oxidative stress and inflammatory injury in chicken livers, J. Trace Elem. Med. Biol. 52 (2019) 118–125, https://doi.org/10.1016/j.jtemb.2018.12.010.
[4] E. Marettová, M. Maretta, J. Legáth, E. Košutzká, The retention of cadmium and selenium influence in fowl and chickens of F1 generation, Biol. Trace Elem. Res. 147 (2012) 130–134, https://doi.org/10.1007/s12011-011-9305-5.
[5] M.V. Varoni, V. Pasciu, S.D. Gadau, E. Baralla, E. Serra, D. Palomba, M.P. Demontis, Possible antioxidant effect of Lycium barbarum polysaccharides on hepatic cadmium-induced oxidative stress in rats, Environ. Sci. Pollut. Control Ser. 24 (2017) 2946–2955, https://doi.org/10.1007/s11356-016-8050-x.
[6] Y. Li, S.C. Lim, Cadmium-induced apoptosis of hepatocytes is not associated with death receptor-related caspase-dependent pathways in the rat, Environ. Toxicol. Pharmacol. 24 (2007) 231–238, https://doi.org/10.1016/j.etap.2007.05.010.
[7] T.N. Pham, M. Marion, F. Denizeau, C. Jumarie, Cadmium-induced apoptosis in rat hepatocytes does not necessarily involve caspase-dependent pathways, Toxicol. Vitro 20 (2006) 1331–1342, https://doi.org/10.1016/j.tiv.2006.05.005.
[8] S. Boucherie, C. Decaens, J.M. Verbavatz, B. Grosse, M. Erard, F. Merola, D. Cassio, L. Combettes, Cadmium disorganises the scaffolding of gap and tight junction proteins in the hepatic cell line WIF B9, Biol Cell 105 (2013) 561–575, https://doi.org/10.1111/boc.201200092.
[9] H. Zou, L. Zhuo, T. Han, D. Hu, X. Yang, Y. Wang, Y. Yuan, J. Gu, J. Bian, X. Liu, Z. Liu, Autophagy and gap junctional intercellular communication inhibition are involved in cadmium-induced apoptosis in rat liver cells, Biochem. Biophys. Res. Commun. 459 (2015) 713–719, https://doi.org/10.1016/j.bbrc.2015.03.027.
[10] J.Z. Zhou, J.X. Jiang, Gap junction and hemichannel-independent actions of connexins on cell and tissue functions–an update, FEBS Lett. 588 (2014) 1186–1192, https://doi.org/10.1016/j.febslet.2014.01.001.
[11] F.F. Zhang, N. Morioka, K. Nakashima-Hisaoka, Y. Nakata, Spinal astrocytes stimulated by tumor necrosis factor-α and/or interferon-γ attenuate connexin 43-gap junction via c-jun terminal kinase activity, J. Neurosci. Res. 91 (2013) 745–756, https://doi.org/10.1002/jnr.23213.
[12] P. Kameritsch, N. Khandoga, U. Pohl, K. Pogoda, Gap junctional communication promotes apoptosis in a connexin-type-dependent manner, Cell Death Dis. 4 (2013) e584, https://doi.org/10.1038/cddis.2013.105.
[13] C.L. Grek, J.M. Rhett, G.S. Ghatnekar, Cardiac to cancer: connecting connexins to clinical opportunity, FEBS Lett. 588 (2014) 1349–1364, https://doi.org/10.1016/j.febslet.2014.02.047.

[14] H. Zou, X. Liu, T. Han, D. Hu, Y. Yuan, J. Gu, J. Bian, Z. Liu, Alpha-lipoic acid protects against cadmium-induced hepatotoxicity via calcium signalling and gap junctional intercellular communication in rat hepatocytes, J. Toxicol. Sci. 40 (2015) 469–477, https://doi.org/10.2131/jts.40.469.
[15] Q. Liu, X. Ji, Z. Ge, H. Diao, X. Chang, L. Wang, Q. Wu, Role of connexin 43 in cadmium-induced proliferation of human prostate epithelial cells, J. Appl. Toxicol. 37 (2017) 933–942, https://doi.org/10.1002/jat.3441.
[16] J.T. Fong, R.M. Kells, A.M. Gumpert, J.Y. Marzillier, M.W. Davidson, M.M. Falk, Internalized gap junctions are degraded by autophagy, Autophagy 8 (2012) 794–811, https://doi.org/10.4161/auto.19390.
[17] D. Glick, S. Barth, K.F. Macleod, Autophagy: cellular and molecular mechanisms, J. Pathol. 221 (2010) 3–12, https://doi.org/10.1002/path.2697.
[18] N. Mizushima, B. Levine, A.M. Cuervo, D.J. Klionsky, Autophagy fights disease through cellular self-digestion, Nature 451 (2008) 1069–1075, https://doi.org/10.1038/nature06639.
[19] M. Condello, E. Pellegrini, M. Caraglia, S. Meschini, Targeting autophagy to overcome human diseases, Int. J. Mol. Sci. 20 (2019), https://doi.org/10.3390/ijms20030725.
[20] X. Song, Z. Li, F. Liu, Z. Wang, L. Wang, Restoration of autophagy by puerarin in lead-exposed primary rat proximal tubular cells via regulating AMPK-mTOR signaling, J. Biochem. Mol. Toxicol. 31 (2017), https://doi.org/10.1002/jbt.21869.
[21] J. Liu, G. Liao, H. Tu, Y. Huang, T. Peng, Y. Xu, X. Chen, Z. Huang, Y. Zhang, X. Meng, F. Zou, A protective role of autophagy in Pb-induced developmental neurotoxicity in zebrafish, Chemosphere 235 (2019) 1050–1058, https://doi.org/10.1016/j.chemosphere.2019.06.227.
[22] H.L. Zhu, X.F. Xu, X.T. Shi, Y.J. Feng, Y.W. Xiong, Y. Nan, C. Zhang, L. Gao, Y.H. Chen, D.X. Xu, H. Wang, Activation of autophagy inhibits cadmium-triggered apoptosis in human placental trophoblasts and mouse placenta, Environ Pollut 254 (2019), 112991, https://doi.org/10.1016/j.envpol.2019.112991.
[23] J. Gu, Z. Ren, J. Zhao, F.A. Peprah, Y. Xie, D. Cheng, Y. Wang, H. Liu, C.K. Chu Wong, Y. Zhou, H. Shi, Calcimimetic compound NPS R-467 protects against chronic cadmium-induced mouse kidney injury by restoring autophagy process, Ecotoxicol. Environ. Saf. 189 (2020), 110052, https://doi.org/10.1016/j.ecoenv.2019.110052.
[24] D.M. Templeton, Y. Liu, Multiple roles of cadmium in cell death and survival, Chem. Biol. Interact. 188 (2010) 267–275, https://doi.org/10.1016/j.cbi.2010.03.040.
[25] M. Chen, X. Li, R. Fan, J. Yang, X. Jin, S. Hamid, S. Xu, Cadmium induces BNIP3-dependent autophagy in chicken spleen by modulating miR-33-AMPK axis, Chemosphere 194 (2018) 396–402, https://doi.org/10.1016/j.chemosphere.2017.12.026.
[26] J.R. Li, Y.C. Ou, C.C. Wu, J.D. Wang, S.Y. Lin, Y.Y. Wang, W.Y. Chen, S.L. Liao, C.J. Chen, Endoplasmic reticulum stress and autophagy contributed to cadmium nephrotoxicity in HK-2 cells and Sprague-Dawley rats, Food Chem. Toxicol. 146 (2020), 111828, https://doi.org/10.1016/j.fct.2020.111828.
[27] A. Lichtenstein, P.J. Minogue, E.C. Beyer, V.M. Berthoud, Autophagy: a pathway that contributes to connexin degradation, J. Cell Sci. 124 (2011) 910–920, https://doi.org/10.1242/jcs.073072.
[28] H. Zou, J. Yuan, Y. Zhang, T. Wang, Y. Chen, Y. Yuan, J. Bian, Z. Liu, Gap junction intercellular communication negatively regulates cadmium-induced autophagy and inhibition of autophagic flux in Buffalo rat liver 3A cells, Front. Pharmacol. 11 (2020), 596046, https://doi.org/10.3389/fphar.2020.596046.
[29] S. Yildirim, F.C. Celikezen, G. Oto, E. Sengul, M. Bulduk, M. Tasdemir, D. Ali Cinar, An investigation of protective effects of litium borate on blood and histopathological parameters in acute cadmium-induced rats, Biol. Trace Elem. Res. 182 (2018) 287–294, https://doi.org/10.1007/s12011-017-1089-9.
[30] Q. Zhai, G. Wang, J. Zhao, X. Liu, F. Tian, H. Zhang, W. Chen, Protective effects of Lactobacillus plantarum CCFM8610 against acute cadmium toxicity in mice, Appl. Environ. Microbiol. 79 (2013) 1508–1515, https://doi.org/10.1128/aem.03417-12.
[31] L. Wang, H. Li, H. Wei, X. Wu, L. Ke, Identification of cadmium-induced Agaricus blazei genes through suppression subtractive hybridization, Food Chem. Toxicol. 63 (2014) 84–90, https://doi.org/10.1016/j.fct.2013.10.036.
[32] S. Djurasevic, A. Jama, N. Jasnic, P. Vujovic, M. Jovanovic, D. Mitic-Culafic, J. Knezevic-Vukcevic, M. Cakic-Milosevic, K. Ilijevic, J. Djordjevic, The protective effects of probiotic bacteria on cadmium toxicity in rats, J. Med. Food 20 (2017) 189–196, https://doi.org/10.1089/jmf.2016.0090.
[33] A. Begic, A. Djuric, M. Ninkovic, I. Stevanovic, D. Djurdjevic, M. Pavlovic, K. Jelic, A. Pantelic, G. Zebic, B. Dejanovic, I. Stanojevic, D. Vojvodic, P. Milosavljevic, M. Djukic, L. Saso, Disulfiram moderately restores impaired hepatic redox status of rats subchronically exposed to cadmium, J Enzyme Inhib Med Chem 32 (2017) 478–489, https://doi.org/10.1080/14756366.2016.1261132.
[34] Z. Ge, H. Diao, M. Yu, X. Ji, Q. Liu, X. Chang, Q. Wu, Connexin 43 mediates changes in protein phosphorylation in HK-2 cells during chronic cadmium exposure, Environ. Toxicol. Pharmacol. 53 (2017) 184–190, https://doi.org/10.1016/j.etap.2017.06.003.
[35] C. Zhang, J. Lin, J. Ge, L.L. Wang, N. Li, X.T. Sun, H.B. Cao, J.L. Li, Selenium triggers Nrf2-mediated protection against cadmium-induced chicken hepatocyte autophagy and apoptosis, Toxicol. Vitro 44 (2017) 349–356, https://doi.org/10.1016/j.tiv.2017.07.027.
[36] X. Zhao, L. Jin, N. Shen, B. Xu, W. Zhang, H. Zhu, Z. Luo, Salidroside inhibits endogenous hydrogen peroxide induced cytotoxicity of endothelial cells, Biol. Pharm. Bull. 36 (2013) 1773–1778, https://doi.org/10.1248/bpb.b13-00406.
[37] K. Du, C.D. Williams, M.R. McGill, Y. Xie, A. Farhood, M. Vinken, H. Jaeschke, The gap junction inhibitor 2-aminoethoxy-diphenyl-borate protects against acetaminophen hepatotoxicity by inhibiting cytochrome P450 enzymes and c-jun N-terminal kinase activation, Toxicol. Appl. Pharmacol. 273 (2013) 484–491, https://doi.org/10.1016/j.taap.2013.09.010.
[38] S.J. Patel, J.M. Milwid, K.R. King, S. Bohr, A. Iracheta-Vellve, M. Li, A. Vitalo, B. Parekkadan, R. Jindal, M.L. Yarmush, Gap junction inhibition prevents drug-induced liver toxicity and fulminant hepatic failure, Nat. Biotechnol. 30 (2012) 179–183, https://doi.org/10.1038/nbt.2089.
[39] A. Rawanduzy, A. Hansen, T.W. Hansen, M. Nedergaard, Effective reduction of infarct volume by gap junction blockade in a rodent model of stroke, J. Neurosurg. 87 (1997) 916–920, https://doi.org/10.3171/jns.1997.87.6.0916.
[40] D. Garcia-Dorado, J. Inserte, M. Ruiz-Meana, M.A. González, J. Solares, M. Juliá, J.A. Barrabés, J. Soler-Soler, Gap junction uncoupler heptanol prevents cell-to-cell progression of hypercontracture and limits necrosis during myocardial reperfusion, Circulation 96 (1997) 3579–3586, https://doi.org/10.1161/01.cir.96.10.3579.
[41] A.M. Orogo, B. Gustafsson, Å, Therapeutic targeting of autophagy: potential and concerns in treating cardiovascular disease, Circ. Res. 116 (2015) 489–503, https://doi.org/10.1161/circresaha.116.303791.
[42] D. Lumkwana, A. du Toit, C. Kinnear, B. Loos, Autophagic flux control in neurodegeneration: progress and precision targeting-Where do we stand? Prog Neurobiol 153 (2017) 64–85, https://doi.org/10.1016/j.pneurobio.2017.03.006.
[43] K. Singh, A. Sharma, M.C. Mir, J.A. Drazba, W.D. Heston, C. Magi-Galluzzi, D. Hansel, B.P. Rubin, E.A. Klein, A. Almasan, Autophagic flux determines cell death and survival in response to Apo2L/TRAIL (dulanermin), Mol. Cancer 13 (2014) 70, https://doi.org/10.1186/1476-4598-13-70.
[44] E. Morel, M. Mehrpour, J. Botti, N. Dupont, A. Hamaï, A.C. Nascimbeni, P. Codogno, Autophagy: a druggable process, Annu. Rev. Pharmacol. Toxicol. 57 (2017) 375–398, https://doi.org/10.1146/annurev-pharmtox-010716-104936.
[45] M. Allaire, P.E. Rautou, P. Codogno, S. Lotersztajn, Autophagy in liver diseases: time for translation? J. Hepatol. 70 (2019) 985–998, https://doi.org/10.1016/j.jhep.2019.01.026.
[46] X.B. Song, G. Liu, F. Liu, Z.G. Yan, Z.Y. Wang, Z.P. Liu, L. Wang, Autophagy blockade and lysosomal membrane permeabilization contribute to lead-induced nephrotoxicity in primary rat proximal tubular cells, Cell Death Dis. 8 (2017), e2863, https://doi.org/10.1038/cddis.2017.262.
[47] H. Zou, T. Wang, J. Yuan, J. Sun, Y. Yuan, J. Gu, X. Liu, J. Bian, Z. Liu, Cadmium-induced cytotoxicity in mouse liver cells is associated with the disruption of autophagic flux via inhibiting the fusion of autophagosomes and lysosomes, Toxicol. Lett. 321 (2020) 32–43, https://doi.org/10.1016/j.toxlet.2019.12.019.
[48] M. Komatsu, S. Waguri, M. Koike, Y.S. Sou, T. Ueno, T. Hara, N. Mizushima, J. Iwata, J. Ezaki, S. Murata, J. Hamazaki, Y. Nishito, S. Iemura, T. Natsume, T. Yanagawa, J. Uwayama, E. Warabi, H. Yoshida, T. Ishii, A. Kobayashi, M. Yamamoto, Z. Yue, Y. Uchiyama, E. Kominami, K. Tanaka, Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice, Cell 131 (2007) 1149–1163, https://doi.org/10.1016/j.cell.2007.10.035.
[49] Y. Yin, G. Sun, E. Li, K. Kiselyov, D. Sun, ER stress and impaired autophagy flux in neuronal degeneration and brain injury, Ageing Res. Rev. 34 (2017) 3–14, https://doi.org/10.1016/j.arr.2016.08.008.
[50] S.R. Yoshii, N. Mizushima, Monitoring and measuring autophagy, Int. J. Mol. Sci. 18 (2017), https://doi.org/10.3390/ijms18091865.

[51] V. Kolluru, D. Pal, A.M.S. Papu John, M.K. Ankem, J.H. Freedman, C. Damodaran, Induction of Plac8 promotes pro-survival function of autophagy in cadmium-induced prostate carcinogenesis, Cancer Lett. 408 (2017) 121–129, https://doi.org/10.1016/j.canlet.2017.08.023.

[52] M. Maes, S. Crespo Yanguas, J. Willebrords, B. Cogliati, M. Vinken, Connexin and pannexin signaling in gastrointestinal and liver disease, Transl. Res. 166 (2015) 332–343, https://doi.org/10.1016/j.trsl.2015.05.005.

[53] M. Vinken, M. Maes, R. Cavill, D. Valkenborg, J.K. Ellis, E. Decrock, L. Leybaert, A. Staes, K. Gevaert, A.G. Oliveira, G.B. Menezes, B. Cogliati, M.L. Dagli, T. M. Ebbels, E. Witters, H.C. Keun, T. Vanhaecke, V. Rogiers, Proteomic and metabolomic responses to connexin43 silencing in primary hepatocyte cultures, Arch. Toxicol. 87 (2013) 883–894, https://doi.org/10.1007/s00204-012-0994-0.