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ORIGINAL ARTICLE

Mitochondrial ALDH2 improves ß-cell survival and function against doxorubicin-induced apoptosis by targeting CK2 signaling

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ABSTRACT

Aims: The aim of this study was to better understand how the chemotherapy drug doxorubicin contributes to the development of β-cell dysfunction and to explore its relationship with mitochondrial aldehyde dehydrogenase-2 (ALDH2).

Materials and Methods: In order to investigate this hypothesis, doxorubicin was administered to INS-1 cells, a rat insulinoma cell line, either with or without several target protein activators and inhibitors. ALDH2 activity was detected with a commercial kit and protein levels were determined with western blot. Mitochondrial ROS, membrane potential, and lipid ROS were determined by commercial fluorescent probes. The cell viability was measured by CCK-assay.

Results: Exposure of INS-1 cells to doxorubicin decreased active insulin signaling resulting in elevated ALDH2 degradation, compared with control cells by the induction of acid sphingomyelinase mediated ceramide induction. Further, ceramide induction potentiated doxorubicin induced mitochondrial dysfunction. Treatment with the ALDH2 agonist, ALDA1, blocked doxorubicin-induced acid sphingomyelinase activation which significantly blocked ceramide induction and mitochondrial dysfunction mediated cell death. Treatment with the ALDH2 agonist, ALDA1, stimulated casein kinase-2 (CK2) mediated insulin signaling activation. CK2 silencing neutralized the function of ALDH2 in the doxorubicin treated INS-1 cells.

Conclusions: Mitochondrial ALDH2 activation could inhibit the progression of doxorubicin induced pancreatic β-cell dysfunction by inhibiting the acid sphingomyelinase induction of ceramide, by regulating the activation of CK2 signaling. Our research lays the foundation of ALDH2 activation as a therapeutic target for the precise treatment of chemotherapy drug induced β-cell dysfunction.

INTRODUCTION

A higher risk of diabetes, which is characterized by impaired glucose-stimulated insulin secretion (GSIS) from pancreatic β--cells and peripheral insulin resistance, has been associated with exposure to chemotherapeutic agents. Conversely, it has been suggested that diabetes may play a role in the development of cancer through hyperglycemia, hyperinsulinemia, and chronic inflammation linked to obesity[1]. Owing to the use of chemotherapy in cancer treatments, numerous studies have demonstrated the link between diabetes and cancer[2–5]. Miyabayashi *et al.*[6] recently reported on a case of type 1 diabetes in a breast cancer patient that was triggered by the chemotherapy drug doxorubicin with impaired insulin secretion. The Food and Drug Administration (FDA) has approved doxorubicin as one of the most effective chemotherapy drugs

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for the treatment of a number of tumors like sarcomas, carcinomas, breast cancers, and hematological malignancies, and it has been shown to have significant therapeutic potential along with adverse effects[7,8]. However, doxorubicin's toxic effects are multidimensional, and its exact mechanisms of action are still largely unclear. It has been demonstrated previously that doxorubicin inhibits rat islet insulin secretion *in vitro* at levels lower than those used in chemotherapy, indicating that it may be a potential target for chemotherapy-induced diabetes[9]. Several studies have shown that doxorubicin can induce cell death by apoptosis which was potentiated by ceramide accumulation[10,11]. Furthermore, it has long been known that ceramide plays a signaling role in both the *in vitro* and *in vivo* inflammatory responses to the establishment of insulin resistance. It has also been reported that ceramide increases the inflammatory response by inducing the production of certain inflammatory proteins[12–14]. Notably, the nature of doxorubicin's cellular signaling that determines pancreatic β-cell death is still unknown.

As a key regulator of mitochondrial energy metabolism and a scavenger of reactive lipid peroxides such as 4-HNE and MDA in the regulation of ROS homeostasis, we concentrated on mitochondrial aldehyde dehydrogenase-2 (ALDH2) to explore the underlying regulatory mechanism. Primarily known for its involvement in the metabolism of ethanol, mitochondrial ALDH2 is linked to a decrease in the build-up of aldehydes inside the mitochondria and hence contributes to the protection of the mitochondria[15,16]. Moreover, doxorubicin exhibit abnormal accumulation of 4-HNE-modified protein adducts from lipid peroxidation, which has been confirmed to be a risk factor and potentially influences oxidative stress during cardiotoxicity. Remarkably, ALDH2 activation shows reduced 4-HNE-modified protein adducts and protein expression due to improved mitochondrial metabolism against doxorubicin induced cardiotoxicity[17,18]. However, the association between ALDH2 and doxorubicin induced oxidative stress in β-cells has not been studied. Therefore, we investigated the role of ALDH2 in the doxorubicin-induced pathogenic impact in pancreatic β-cells, which is linked to cell death. In particular, understanding β-cell dysfunction and developing effective target-specific treatment methods will be aided by finding efficient target-specific downstream signaling.

MATERIALS AND METHODS

Cell culture and chemicals

The rat insulinoma cell line (INS-1) was cultured in RPMI 1640 (Life Technologies, Inc., NY, USA) media supplemented with 10% fetal bovine serum (FBS) (Gibco, NY, USA), 100 units/mL penicillin, and 100 μg/mL streptomycin at 37°C in a 5% CO_2 environment. The cells were exposed to doxorubicin (1–2 μM) or C2-ceramide (50 μM) for the indicated time intervals. ALDA1 (25 μM), CX-4945 (10 μM), or vehicle (0.1% DMSO) was added to the cells 2 h before treatment. Reagents were sourced commercially as follows: Doxorubicin, ALDA1, C2-Ceramide (Sigma-Aldrich, MO, USA; K); and CX-4945 from SelleckChem. The percentage of viable cells was determined using a cell counting kit-8 (CCK-8; Dojindo Lab., Kumamoto, Japan).

Mitochondrial functional assay

The generation of mitochondrial superoxide was measured using the mitochondrial superoxide indicator MitoSOX red (Molecular Probes). Briefly, after incubation under indicated experimental conditions, the treated cells were incubated with 5 μM mitoSOX red for 10 min at 37°C in the dark. After washing the cells, the fluorescence (mitoSOX: ~510/580 nm) was recorded using a Molecular Device fluorescent plate reader. A TMRE assay kit (#ab113852; Abcam) was used to measure the mitochondrial membrane potential in accordance with the manufacturer's instructions. Fluorescent signals were recorded on a fluorescent microplate reader (Molecular Devices) at excitation and emission wavelengths of 549 and 575 nm, respectively, after the cells were stained with TMRE (200 nM) for 30 min and then rinsed with PBS. The dye BODIPY 581/591 C11 (Thermo Fisher Scientific, MA, USA) was used for the lipid peroxidation assay. After being incubated under the specified experimental conditions, the cells were stained for 30 min at 37°C using 1 μM BODIPY 581/591 C11 dye (Thermo Fisher Scientific, MA, USA). They were then rinsed twice with PBS and visualized under a fluorescence microscope. To quantify lipid ROS, the mean fluorescence intensity was used. Following the experiment, an aldehyde dehydrogenase (ALDH2) activity assay kit (ab115348; Abcam) was utilized in accordance with the manufacturer's instructions to determine the ALDH2 activity by measuring the absorbance at 450 nm.

Western blotting

Following resolution of the cell protein lysates on an Invitrogen NuPAGE 4–12% Bis-Tris gel, the samples were transferred onto PVDF (polyvinylidene fluoride) membranes (Millipore, Billerica, MA, USA). Following blocking, the membranes were gently rocked with the following primary antibodies at 4°C: P-AKT (473,308) (1:1,000), AKT (1:1,000), CK2α (1:1,000) cleaved caspase 3 (1:1,000) (Cell Signaling Technology, Danvers, MA, USA); cytochrome c (1:2,000) (BD Biosciences, San Jose, CA, USA); P-AKT (129) (1:1,000), ALDH2 (1:1,000), GPX4 (1:1,000) γH2AX (1:1,000), and ACTIN (1:5,000) (Abcam, Cambridge, UK); SMPD1 (1:1,000) (LSBio, WA,USA). After that, the membranes were washed and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies. Enhanced chemiluminescence (ECL) reagents (ECL Plus; Amersham, GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK) were used to identify immunoreactive proteins.

Statistical analysis

Student's *t*-test was used to identify differences that were statistically significant. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Doxorubicin inhibits insulin signaling and induces mitochondrial dysfunction

To identify whether doxorubicin induces β-cell death by inhibiting insulin signaling, INS-1 cells were exposed to doxorubicin at different doses and subjected to immunoblotting studies. Our results reveal that doxorubicin treatment results in decreased AKT signaling, as evidenced by decreased phosphorylation of AKT at Ser473 and Thr308 active sites (Figure 1a). We next questioned if protein kinase CK2, a multipotential serine/threonine kinase implicated in the regulation of numerous distinct cellular signaling pathways, might also be involved in the regulation of insulin signaling in doxorubicin treated β-cells. Phosphorylation of AKT at Ser129 by CK2 enhances cell

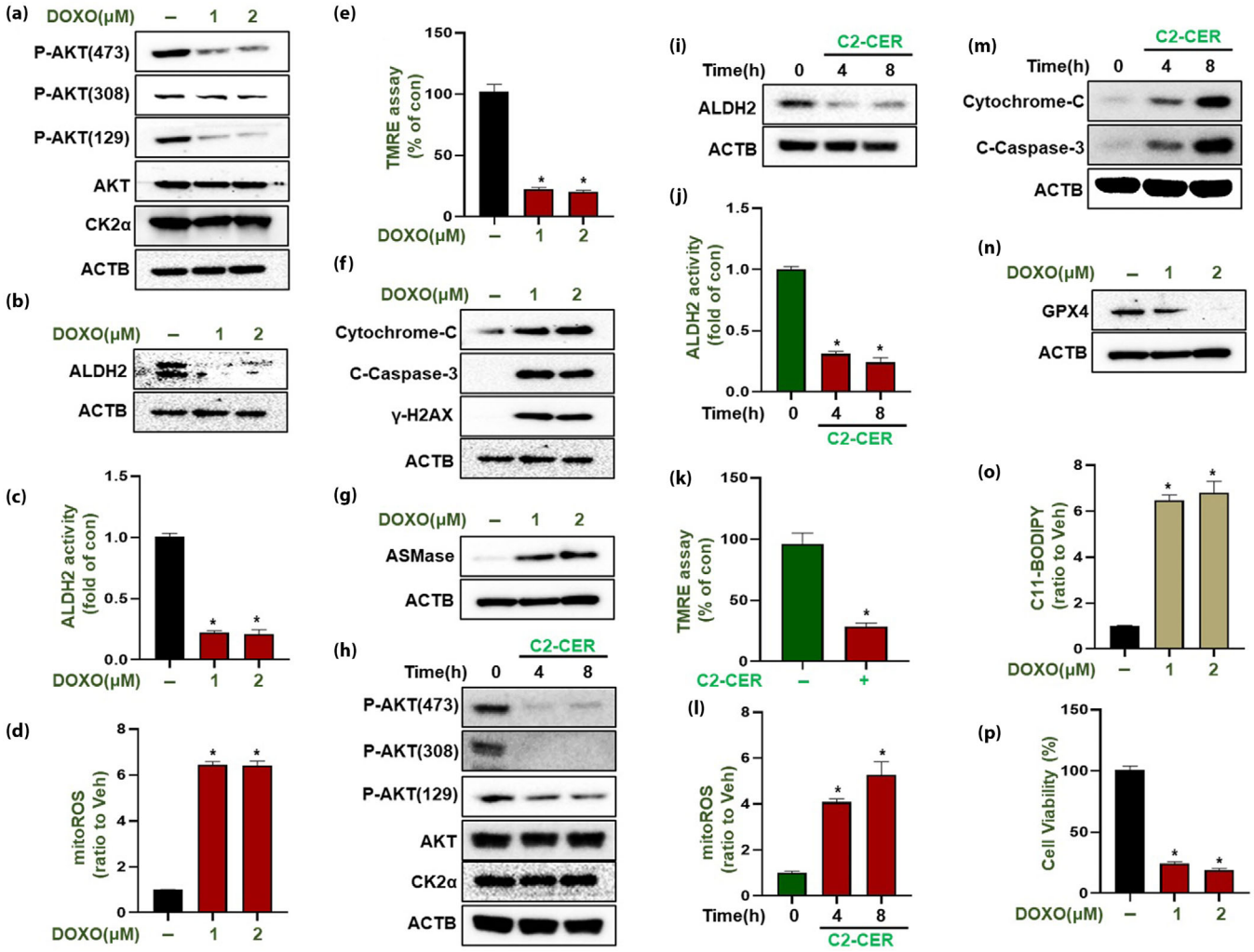

Figure 1 | Doxorubicin inhibits insulin signaling and induces mitochondrial dysfunction. (a, b) The INS-1 cells were treated with doxorubicin (1–2 μM) for 12 h and then AKT phosphorylation, ALDH2 protein levels were analyzed by western blotting. (c) ALDH2 activity was measured as described in the Materials and Methods section (*P < 0.005 vs Control). (d, e) Cells were treated with doxorubicin (1–2 μM) for 12 h and mitoROS and mitochondrial membrane potential was analyzed by mitoSOX and TMRE Assay as per the manufacturer's instruction (*P < 0.001 vs Control). (f, g) Cytosolic cytochrome c, cleaved caspase-3, γH2AX, and acid sphingomyelinase protein levels were quantified by immunoblotting. (h, i) The INS-1 cells were treated with C2-ceramide (50 μM) for the indicated time periods. Western blots of P-AKT and ALDH2 are shown. (j) Measurement of ALDH2 activity in INS-1 cells after C2-ceramide (50 μM) treatment (*P < 0.005 vs Control). (k, l) Relative measurement of mitoROS and mitochondrial membrane potential in C2-ceramide (50 μM) treated cells (*P < 0.005 vs Control). (m) Cytosolic cytochrome c, cleaved caspase-3 and protein levels were quantified by immunoblotting. (n, o) Measurement of GPX4 protein level and lipid peroxidation in INS-1 cells after doxorubicin (1–2 μM) treatment using western blot and C11 BODIPY dye (*P < 0.001 vs Control). (p) INS-1 cells were treated with doxorubicin (1–2 μM) for 12 h, and cell viability was analyzed by CCK assay (*P < 0.001 vs control). All data are expressed as the mean ± SD of at least three independent experiments.

viability by generating constitutively active AKT, an essential priming mediator in the pro-survival signaling cascade of insulin signaling[19]. Interestingly, doxorubicin treatment resulted in a decrease in casein kinase-2 (CK2) mediated AKT ser129 phosphorylation (Figure 1a). Under the same conditions, we observed increased degradation of mitochondrial ALDH2 which contributes to increased lipid peroxidation during apoptosis (Figure 1b). Notably, doxorubicin treatment also reduced ALDH2 activity (Figure 1c). To assess whether the effects of doxorubicin on β-cells were mediated by mitochondrial dysfunction, we measured the levels of mitochondrial ROS and membrane potential post doxorubicin exposure. Our results show that doxorubicin exposure significantly increases the mitochondrial ROS production and membrane potential loss with increased cytochrome-c mediated cleaved caspase-3 induction, as well as the DNA damage marker γH2AX which plays a major role in pancreatic β-cell damage (Figure 1d, f). It is well established that doxorubicin contributes to an increase in ceramide levels and potentiates doxorubicin-induced proapoptotic effects[10,11]. Furthermore, doxorubicin sensitivity and the susceptibility to inhibit cancer cell death are mediated by increased activation of acid sphingomyelinase with a marked release of ceramide[20]. To assess whether the effects of doxorubicin on β--cell dysfunction were mediated by acid sphingomyelinase, we measured the levels of acid sphingomyelinase post doxorubicin exposure by western blotting. Interestingly, our results show that doxorubicin exposure significantly increases acid sphingomyelinase expression (Figure 1g). In order to determine the relevance of ceramide during doxorubicin toxicity, we exposed INS-1 cells to C2-ceramide (N-acetyl-sphingosine), a biologically active, cell permeable ceramide analog. Interestingly, ceramide treatment potently inhibited insulin signaling, as evidenced by reduced phosphorylation of AKT at Ser473 and Thr308 active sites as well as the AKT Ser129 priming site (Figure 1h). Furthermore, C2-ceramide treatment resulted in a decrease in ALDH2 expression as well as its activity (Figure 1i, j). Under the same conditions, we observed mitochondrial membrane potential loss along with mitoROS production which contribute to cytochrome-c release and cleaved caspase-3 activation (Figure 1k,m). Moreover, reduction of ALDH2 activity led to increased sensitivity of β-cells to ferroptotic-like death by increasing GPX4 degradation (Figure 1n). Moreover, reduction of GPX4 led to an increased sensitivity of β-cells to ferroptotic-like death by increasing lipid ROS production (Figure 1o). As expected, treatment with doxorubicin resulted in decreased cell viability (Figure 1p).

ALDH2 activation inhibits doxorubicin induced mitochondrial dysfunction

To identify whether ALDH2 activation restores β-cell survival under doxorubicin induced oxidative stress, we exposed INS-1 cells to doxorubicin in the presence or absence of ALDA1, an ALDH2 specific agonist. As expected, under doxorubicin-treated cells, ALDA1 administration significantly increased ALDH2 activity (Figure 2a). Interestingly, ALDH2 activation potently reversed doxorubicin-induced inactivation of insulin signaling, as evidenced by enhanced phosphorylation of AKT at Ser473 and Thr308 active sites as well as the AKT Ser129 priming site (Figure 2b). Furthermore, ALDH2 activation reduced mitochondrial ROS production and restored the mitochondrial membrane potential resulting in the prevention of cytochrome-c release and cleaved caspase-3 activation as well as the DNA damage marker γH2AX (Figure 2c,e). In order to ascertain whether ALDH2 activation reverses the doxorubicin induced acid sphingomyelinase, we looked at the expression of acid sphingomyelinase in cells that had been exposed to ALDA1. Remarkably, our results demonstrated that doxorubicin-induced acid sphingomyelinase protein levels were reduced upon treatment of INS-1 cells with the ALDH2 activator, indicating that ALDH2 activation might affect the acid sphingomyelinase induced ceramide generation (Figure 2f). To establish a functional relationship between ALDH2 and ceramide, we assessed the impact of ALDA1 on C2-ceramide treated cells. As expected, the treatment of INS-1 cells with ALDA1 caused a dramatic increase in the ALDH2 activity in response to C2-ceramide treatment (Figure 2g). Prominently, ALDH2 activation resulted in a potent and significant reduction of mitochondrial ROS and an improvement of membrane potential in C2-ceramide-treated cells (Figure 2h,i). Further analyses revealed a significant inhibition of cytochrome c release and caspase-3 activation in C2-ceramide-treated cells in the presence of the ALDH2 agonist ALDA1 (Figure 2j), confirming that the protective effect of ALDA1 on β-cell survival was dependent on ALDH2 activity. Subsequently, we investigated whether ALDH2 activation alters GPX4 expression in doxorubicin-treated cells. Remarkably, our results demonstrated that doxorubicin-induced reduction in GPX4 protein levels was restored upon treatment of INS-1 cells with the ALDH2 activator ALDA1, along with a reduction of lipidROS indicating that ALDH2 activation prevents ferroptotic-like death of β-cells (Figure 2k,l). To a similar extent, activation of ALDH2 with ALDA1 significantly increased cell survival in doxorubicin and C2-ceramide-treated β-cells (Figure 2m,n). Our data demonstrate that the ALDH2-stimulated CK2-AKT signaling axis is intricately involved in the regulation of mitochondrial function in β-cells under doxorubicin treated conditions.

CK2 inhibition reverses ALDH2 protective effect in β-cells

We hypothesized that inhibition of CK2 might impair the ALDH2 protective effects against doxorubicin on β-cells. Crucially, treatment with the CK2 specific inhibitor CX-4945 under doxorubicin and C2-ceramide conditions significantly reversed the effect of ALDH2 on AKT signaling (Figure 3a,b). Consistently, CK2 inhibition was correlated with an increase in mitoROS production in ALDH2 treated cells with a parallel loss of mitochondrial membrane potential (Figure 3c,d). As anticipated, cells treated with CX-4945 had higher levels of cleaved caspase-3 in C2-ceramide treated cells (Figure 3e). Notably,

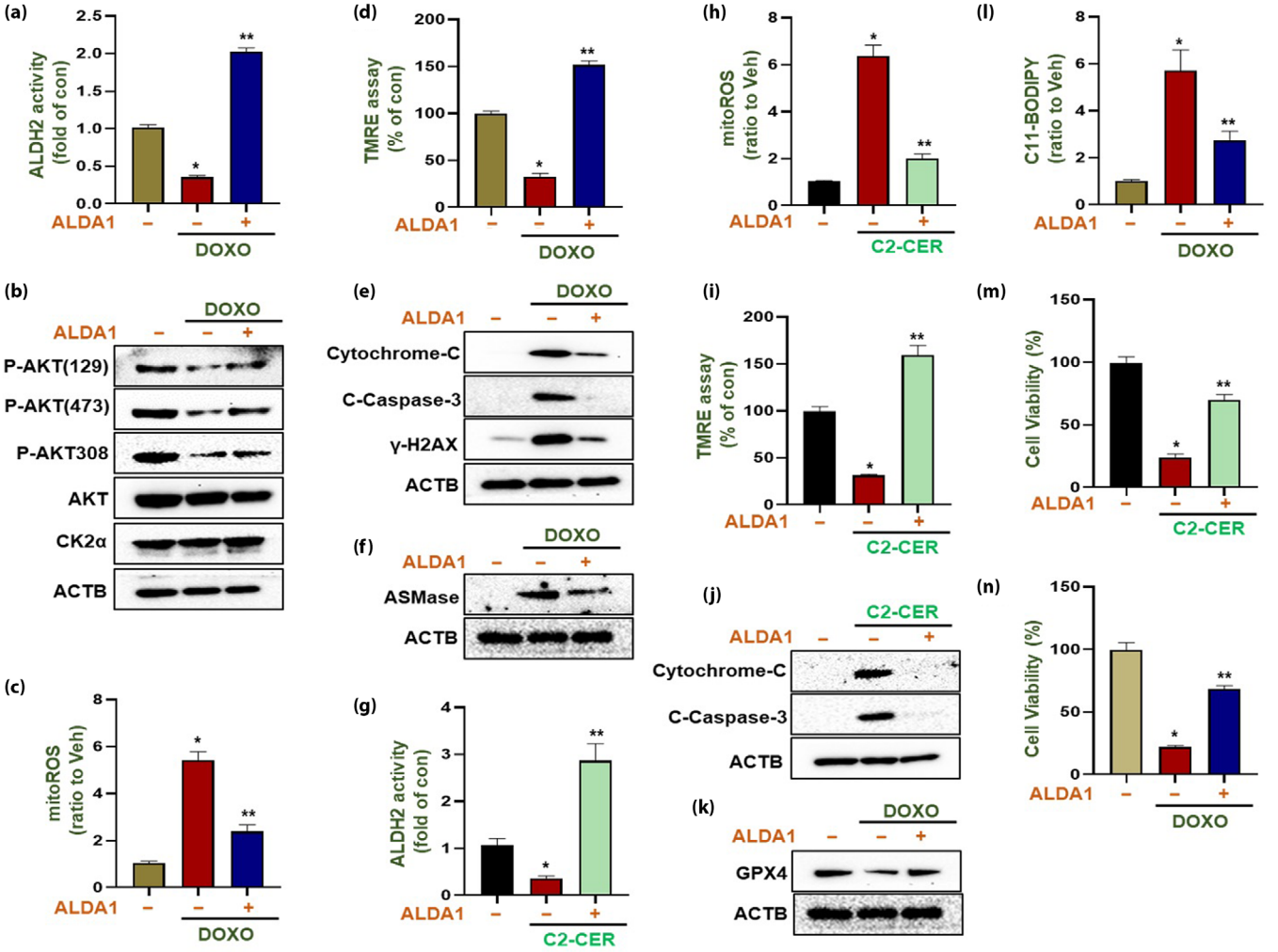

Figure 2 | ALDH2 activation inhibits doxorubicin induced mitochondrial dysfunction. (a) The INS-1 cells were treated with doxorubicin (1 μM) with or without ALDA1 (25 μM) for 12 h (*P < 0.001 vs Control; **P < 0.005 vs DOXO). (b) AKT phosphorylation was quantified by immunoblotting. (c, d) The INS-1 cells were treated with doxorubicin (1 μM) with or without ALDA1 (25 μM) for 12 h (*P < 0.001 vs Control; **P < 0.005 vs DOXO). (e) cytochrome-c, cleaved caspase-3, and DNA damage marker γH2AX protein levels were quantified by immunoblotting. (f) Acid sphingomyelinase protein levels were quantified by immunoblotting. (g) The INS-1 cells were treated with C2-ceramide (50 μM) with or without ALDA1 (25 μM) for 8 h (*P < 0.005 vs Control; **P < 0.001 vs C2-CER). (h–j) Measurement of mitoROS and mitochondrial membrane potential in INS-1 cells after C2-ceramide (50 μM) treatment with or without ALDA1 (25 μM) for 8 h (*P < 0.005 vs Control; **P < 0.001 vs STZ; C2-CER). (j) Cytosolic cytochrome c and cleaved caspase-3 protein levels were quantified by immunoblotting. (k) GPX4 protein levels were measured through immunoblotting. (l) Measurement of lipid peroxidation in ALDA1 (25 μM) treated cells (*P < 0.005 vs Control; **P < 0.001 vs DOXO). (m, n) Cells were treated as mentioned above, and cell viability was analyzed by CCK assay (*P < 0.005 vs Control; **P < 0.001 vs C2-CER; DOXO).

inhibition of CK2 by CX-4945 reversed the ALDH2 effect on cleaved caspase-3 and the DNA damage marker γH2AX in doxorubicin-treated β-cells (Figure 3f). We next investigated whether enhanced GPX4 protein levels could alter lipid ROS upon CK2 inhibition. Our results suggest that CK2 inhibition results in decreased GPX4 stability with increased lipid ROS production (Figure 3g,h). Further analyses revealed a parallel reduction of cell viability in C2-ceramide and doxorubicin-treated cells in the presence of the CK2 inhibitor, confirming that the protective effect of ALDH2 on β-cell apoptosis was dependent on CK2-mediated insulin signaling (Figure 3i,j). Overall, these data indicate that doxorubicin selectively reduces insulin signaling through ceramide in β-cells associated with the reduction of ALDH2, leading to dysfunctional mitochondria-mediated ferroptotic-like death of pancreatic β-cells. Notably, activation of ALDH2-driven CK2-AKT signaling rescues the pancreatic β-cells from ferroptotic-like death by doxorubicin.

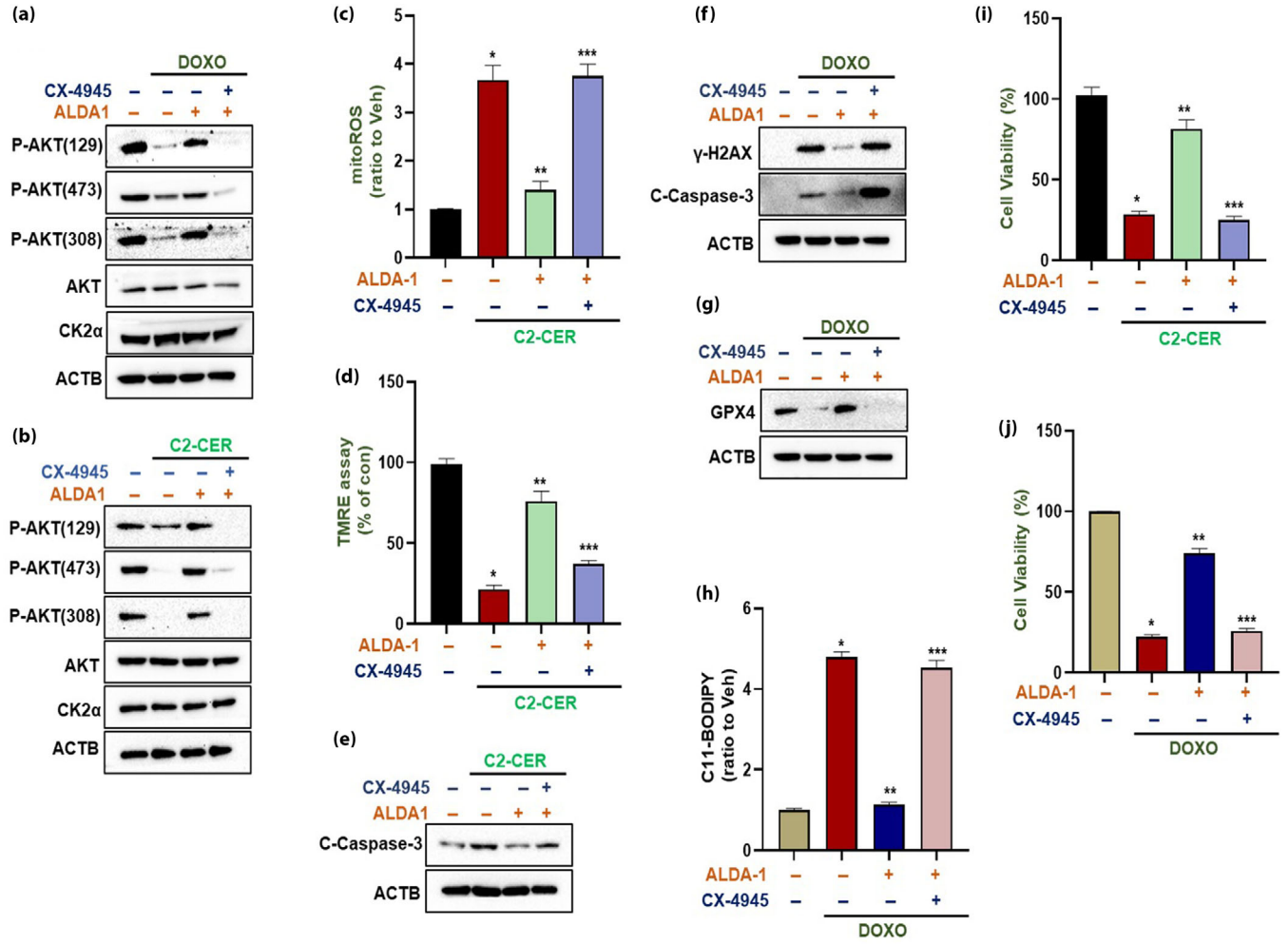

Figure 3 | CK2 inhibition reverses ALDH2 protective effect in β-cells. (a) INS-1 cells were treated with doxorubicin (1 μM) along with ALDA1 (25 μM) for 12 h with or without the CK2 inhibitor CX-4945 (5 μM). (b–e) INS-1 cells were treated with C2-ceramide (50 μM) along with ALDA1 (25 μM) for 8 h with or without the CK2 inhibitor CX-4945 (5 μM) (*$P < 0.001$ vs Control; **$P < 0.005$ vs C2-CER; ***$P < 0.001$ vs CX-4945). (f-h) INS-1 cells were treated with doxorubicin (1 μM) along with ALDA1 (25 μM) for 12 h with or without the CK2 inhibitor CX-4945 (5 μM). The cleaved caspase-3, γH2AX and GPX4 protein levels were quantified by immunoblotting. Measurement of lipid ROS levels by C11 BODIPY dye (*$P < 0.001$ vs Control; **$P < 0.001$ vs DOXO; ***$P < 0.001$ vs CX-4945). (i, j) Cells were treated as mentioned above, and cell viability was analyzed by CCK assay (*$P < 0.001$ vs Control; **$P < 0.005$ vs C2-CER; DOXO ***$P < 0.001$ vs CX-4945). Data are represented as mean ± SD of three independent experiments.

DISCUSSION

This paper presents the first evidence that ALDH2 activation enhances the CK2-driven AKT signaling, which reduces doxorubicin-induced ceramide mediated mitochondrial damage in β-cells. Diabetes and other autoimmune endocrine disorders have been observed to develop in patients taking chemotherapy drugs[2–5]. Furthermore, a number of epidemiological studies have shown the metabolic connections between diabetes and cancer which is associated with insulin resistance and malfunctioning pancreatic β-cells[21–23]. Doxorubicin is one of the most effective chemotherapy medications that has been approved by the Food and Drug Administration (FDA) and it has been shown to have significant therapeutic potential along with adverse effects. Nevertheless, doxorubicin has a variety of harmful effects, and its precise modes of action are still mostly unknown. However, doxorubicin is still often utilized in cancer treatment despite the negative consequences it causes, usually in the form of novel formulations or medication combinations[24,25]. A previous study has demonstrated that doxorubicin inhibits rat islet insulin secretion *in vitro* at dosages lower than those used in chemotherapy by an unknown mechanism[9]. Given the pleiotropic actions of doxorubicin in pathophysiological settings, we propose that aberrant ALDH2 signaling may reduce β-cell survival under doxorubicin-induced oxidative stress by interfering with the downregulation of insulin signaling. ALDH2, a mitochondrial matrix enzyme encoded

in the nucleus, reduces the oxidative stress that acetaldehyde and its metabolites inflict on cells by degrading the metabolite 4-HNE. In addition, doxorubicin causes aberrant accumulation of 4-HNE-modified protein adducts from lipid peroxidation, which has been linked to risk and may potentially influence the oxidative stress in the context of cardiotoxicity[17,18]. Our results have also shown that doxorubicin lowers ALDH2 protein and activity which drives β-cell damage. Furthermore, it has been reported that doxorubicin exposure causes ceramide accumulation due to the action of acid sphingomyelinase, which ultimately results in the death of cancer cells[20]. In support of these reports, our results have also shown that doxorubicin induces acid sphingomyelinase which allows for pro-apoptotic signaling by enhanced ceramide generation. Therefore, we reasoned that increased ceramide generation under doxorubicin conditions would have an adverse effect on ALDH2 expression and its activity. In this context, cells treated with C2-ceramide have been shown to exhibit a decrease in the ALDH2 activity, resulting in a more oxidized environment and cell death. Specifically, a decrease in ALDH2 activity can lead to lipid ROS dependent accumulation of phospholipid hydroperoxides, making β-cells susceptible to ferroptotic-like apoptosis. As the findings support a role of mitochondria mediated ferroptotic-like death of pancreatic β-cells, we have also investigated whether ALDH2 inhibition contributes to the damage of pancreatic β-cells in the oxidative stress response by inhibiting insulin signaling. Besides, insulin signaling is necessary for healthy β-cell activity and several reports have clearly shown that ceramide inhibits the AKT signaling by PP2A activation[26,27]. Notably, we have found the suppression of endogenous insulin signaling upon doxorubicin and ceramide exposure correlates with cell apoptosis. CK2 activation was found to be closely associated with the inhibition of PP2A, resulting in an increase in the phosphorylation of AKT at Ser473 and Thr308 with an enhanced catalytic activity and cell survival[28–30]. However, the metabolic connections of ALDH2 and CK2 in β-cells remain unknown. Interestingly, ALDH2 activation by ALDA1 blocks doxorubicin and ceramide effects on insulin signaling by stimulating CK2, resulting in hyper-activation of AKT Ser129 phosphorylation, which correlates with a further increase in catalytic activity by preventing the dephosphorylation of AKT at Ser473 and Thr308 via inhibiting PP2A. In addition, activation of AKT inhibits early apoptotic events by maintaining mitochondrial integrity[31].We have reported previously that activation of mitochondrial p66Shc signaling may enhance the release of pro-apoptotic factors which may contribute to the induction of apoptosis by ceramide[32]. Most strikingly, CK2 activation was closely associated with the suppression of lipid ROS production upon ALDH2 activation by enhancing GPX4 stability. In line with these findings, we observed a reduction in the generation of lipid reactive oxygen

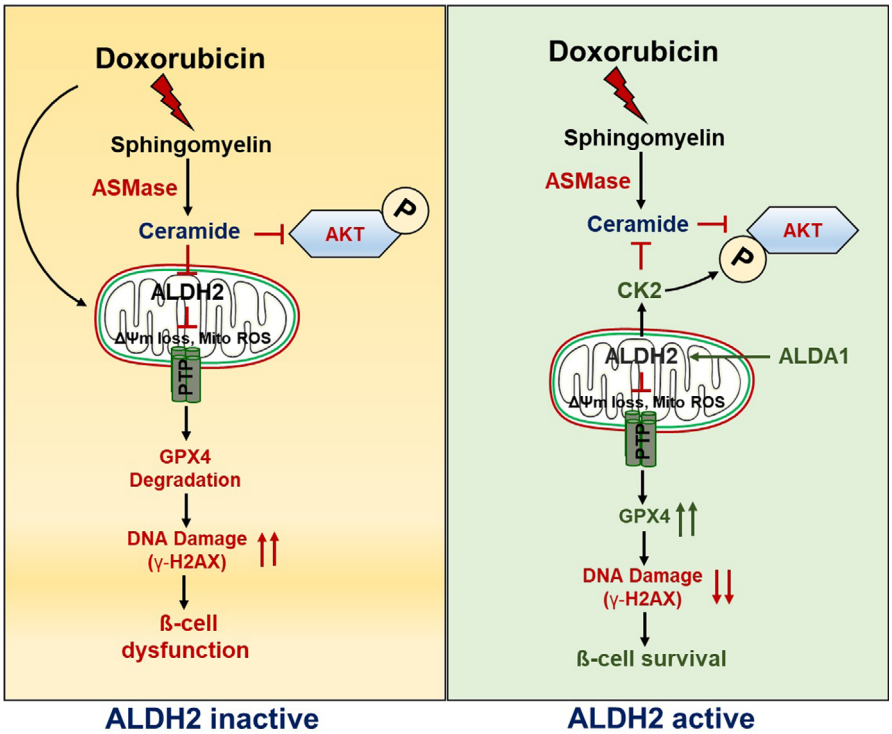

Figure 4 | The activation of mitochondrial ALDH2 improves the CK2-driven AKT signaling, which lessens the mitochondrial damage caused by doxorubicin-induced ceramide in β-cells.

species (ROS) in cells treated with ALDH2 activator, suggesting that ALDH2 plays a role in mediating β-cell survival in doxorubicin-treated conditions. Importantly, inhibition of CK2 using CX-4945, a selective and potent inhibitor of CK2 blocks the ALDH2 induced AKT activity, suggesting that CK2 is directly involved in the ALDH2 induced cell survival in response to doxorubicin and ceramide. We next sought to determine whether the inhibition of CK2 changes the effect of ALDH2 in mitochondrial function in response to doxorubicin. Crucially, treatment with CX-4945, significantly reversed the effect of ALDH2 on mitochondrial function coupling with the release of cytochrome c and caspase-3 activation. In particular, a reduction in GPX4 levels may cause phospholipid hydroperoxides to accumulate in a ROS-dependent manner, which leaves pancreatic β-cells vulnerable to ferroptotic-like cell death. Correspondingly, our data show that the CK2 inhibitor CX-4945 significantly increases lipid ROS production and supports the contention that CK2 activation is indispensable for ALDH2-induced protection from ferroptotic-like death of pancreatic β-cells (Figure 4).

Here, we have presented the first evidence that ceramide produced by doxorubicin causes β-cell failure by blocking CK2-AKT signaling and causing mitochondrial ALDH2 degradation. ALDH2 activation enhances CK2-AKT signaling, which maintains the redox balance and reduces oxidative stress to increase β-cell survival and function. Thus, ALDH2 activation could be a target for novel treatments of diabetes caused by chemotherapy drugs.

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DISCLOSURE

The authors declare no conflict of interest.
Approval of the research protocol: N/A.
Informed consent: N/A.
Registry and the registration no. of the study/trial: N/A.
Animal studies: N/A.

REFERENCES

1. Sciacca L, Vigneri R, Tumminia A, *et al*. Clinical and molecular mechanisms favoring cancer initiation and progression in diabetic patients. *Nutr Metab Cardiovasc Dis* 2013; 23: 808–815.
2. Chen X, Affinati AH, Lee Y, *et al*. Immune checkpoint inhibitors and risk of type 1 diabetes. *Diabetes Care* 2022; 45: 1170–1176.
3. Feng JP, Yuan XL, Li M, *et al*. Secondary diabetes associated with 5-fluorouracil-based chemotherapy regimens in non-diabetic patients with colorectal cancer: Results from a single-centre cohort study. *Colorectal Dis* 2013; 15: 27–33.
4. Dispenzieri A, Loprinzi CL. Chemotherapy-induced insulin-dependent diabetes mellitus. *J Clin Oncol* 1997; 15: 1287.
5. Juanjuan L, Wen W, Zhongfen L, *et al*. Clinical pathological characteristics of breast cancer patients with secondary diabetes after systemic therapy: A retrospective multicenter study. *Tumour Biol* 2015; 36: 6939–6947.
6. Miyabayashi M, Onishi S, Yoshida T, *et al*. A case of doxorubicin and cyclophosphamide therapy-induced type 1 diabetes: A case report. *J Med Case Reports* 2023; 17: 26.
7. Mattioli R, Ilari A, Colotti B, *et al*. Doxorubicin and other anthracyclines in cancers: Activity, chemoresistance and its overcoming. *Mol Aspects Med* 2023; 93: 101205.
8. Kciuk M, Gielecińska A, Mujwar S, *et al*. Doxorubicin-an agent with multiple mechanisms of anticancer activity. *Cells* 2023; 12: 659.
9. Deleers M, Goormaghtigh E. Adriamycin effects on insulin secretion, Ca2+ movements and glucose oxidation in pancreatic islet cells. *Pharmacol Res Commun* 1985; 17: 227–232.
10. Delpy E, Hatem SN, Andrieu N, *et al*. Doxorubicin induces slow ceramide accumulation and late apoptosis in cultured adult rat ventricular myocytes. *Cardiovasc Res* 1999; 43: 398–407.
11. Martínez R, Navarro R, Lacort M, *et al*. Doxorubicin induces ceramide and diacylglycerol accumulation in rat hepatocytes through independent routes. *Toxicol Lett* 2009; 190: 86–90.
12. Meikle PJ, Summers SA. Sphingolipids and phospholipids in insulin resistance and related metabolic disorders. *Nat Rev Endocrinol* 2017; 13: 79–91.
13. Wallace M, Morris C, O'Grada CM, *et al*. Relationship between the lipidome, inflammatory markers and insulin resistance. *Mol Biosyst* 2014; 10: 1586–1595.
14. Magnan C, Le Stunff H. Role of hypothalamic de novo ceramides synthesis in obesity and associated metabolic disorders. *Mol Metab* 2021; 53: 101298.
15. Isse T, Matsuno K, Oyama T, *et al*. Aldehyde dehydrogenase 2 gene targeting mouse lacking enzyme activity shows high acetaldehyde level in blood, brain, and liver after ethanol gavages. *Alcohol Clin Exp Res* 2005; 29: 1959–1964.
16. Isse T, Oyama T, Kitagawa K, *et al*. Diminished alcohol preference in transgenic mice lacking aldehyde dehydrogenase activity. *Pharmacogenetics* 2002; 12: 621–626.
17. Ge W, Yuan M, Ceylan AF, *et al*. Mitochondrial aldehyde dehydrogenase protects against doxorubicin cardiotoxicity through a transient receptor potential channel vanilloid 1-mediated mechanism. *Biochim Biophys Acta* 2016; 1862: 622–634.
18. da Cunha Menezes Souza L, Fernandes FH, Presti PT, *et al*. Effect of doxorubicin on cardiac lipid metabolism-related transcriptome and the protective activity of Alda-1. *Eur J Pharmacol* 2021; 898: 173955.

19. Litchfield DW. Protein kinase CK2: Structure, regulation and role in cellular decisions of life and death. *Biochem J* 2003; 369(Pt 1): 1–15.
20. Grammatikos G, Teichgräber V, Carpinteiro A, *et al*. Overexpression of acid sphingomyelinase sensitizes glioma cells to chemotherapy. *Antioxid Redox Signal* 2007; 9: 1449–1456.
21. Suh S, Kim KW. Diabetes and cancer: Is diabetes causally related to cancer. *Diabetes Metab J* 2011; 35: 193–198.
22. Ryu TY, Park J, Scherer PE. Hyperglycemia as a risk factor for cancer progression. *Diabetes Metab J* 2014; 38: 330–336.
23. Olatunde A, Nigam M, Singh RK, *et al*. Cancer and diabetes: The interlinking metabolic pathways and repurposing actions of antidiabetic drugs. *Cancer Cell Int* 2021; 21: 499.
24. Elfadadny A, Ragab RF, Hamada R, *et al*. Natural bioactive compounds-doxorubicin combinations targeting topoisomerase II-alpha: Anticancer efficacy and safety. *Toxicol Appl Pharmacol* 2023; 461: 116405.
25. Qiu C, Wu Y, Shi Q, *et al*. Advanced strategies for nucleic acids and small-molecular drugs in combined anticancer therapy. *Int J Biol Sci* 2023; 19: 789–810.
26. Summers SA. Ceramides in insulin resistance and lipotoxicity. *Prog Lipid Res* 2006; 45: 42–72.
27. Wolff RA, Dobrowsky RT, Bielawska A, *et al*. Role of ceramide-activated protein phosphatase in ceramide-mediated signal transduction. *J Biol Chem* 1994; 269: 19605–19609.
28. Di Maira G, Salvi M, Arrigoni G, *et al*. Protein kinase CK2 phosphorylates and upregulates Akt/PKB. *Cell Death Differ* 2005; 12: 668–677.
29. Di Maira G, Brustolon F, Pinna LA, *et al*. Dephosphorylation and inactivation of Akt/PKB is counteracted by protein kinase CK2 in HEK 293T cells. *Cell Mol Life Sci* 2009; 66: 3363–3373.
30. Ponce DP, Yefi R, Cabello P, *et al*. CK2 functionally interacts with AKT/PKB to promote the β-catenin-dependent expression of survivin and enhance cell survival. *Mol Cell Biochem* 2011; 356: 127–132.
31. Gottlob K, Majewski N, Kennedy S, *et al*. Inhibition of early apoptotic events by Akt/PKB is dependent on the first committed step of glycolysis and mitochondrial hexokinase. *Genes Dev* 2001; 15: 1406–1418.
32. Karunakaran U, Elumalai S, Moon JS, *et al*. CD36 dependent redoxosomes promotes ceramide-mediated pancreatic β-cell failure via p66Shc activation. *Free Radic Biol Med* 2019; 134: 505–515.