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Original Article

Niclosamide: drug repurposing for human chondrosarcoma treatment via the caspase-dependent mitochondrial apoptotic pathway

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Abstract: Poor sensitivity to chemotherapy drugs and high recurrence rates are the bottlenecks to successful chondrosarcoma treatment. Notably, niclosamide has been identified as a potential anti-cancer agent. To investigate the effects and mechanisms of niclosamide in the context of human chondrosarcoma treatment, SW1353 and CAL78 human chondrosarcoma cells were treated with various concentrations of niclosamide. The CKK-8 assay was performed to quantify cell viability. Cell proliferation was determined with crystal violet staining and colony forming assays. TUNEL and annexin V-FITC flow cytometry assays were performed to detect cell apoptosis. Wound healing and Transwell assays were conducted to evaluate migratory and invasive cell behaviors. The effect of niclosamide on the mitochondria was evaluated with the JC-1 and Seahorse Cell Mito Stress Assays. The expression of caspase-3, cleaved caspase-3, caspase-9, cleaved caspase-9, and β-tubulin levels were investigated by western blotting. Collectively, the data demonstrated that niclosamide inhibited cell growth and proliferation, attenuated migratory and invasive cell behaviors, and promoted apoptosis. Niclosamide is as a potent chondrosarcoma tumor inhibitor that activates the caspase-dependent mitochondrial apoptotic pathway and could be a novel therapeutic approach to treat chondrosarcoma.

Keywords: Drug repurposing, niclosamide, tumor inhibitor, human chondrosarcoma, mitochondrial apoptosis, mitochondrial uncoupling

Introduction

Chondrosarcoma (CHS), which is a cartilage-forming bone tumor, is the third most common malignant tumor in humans. Chondrosarcoma incorporates a heterogeneous group of malignant tumors that exhibit hyaline cartilage differentiation and is associated with poor clinical outcomes and radiation chemo-resistance [1-3]. Drug repurposing, which is a strategy where new uses for approved drugs are identified, has been a rapid and effective way to develop new anti-cancer agents. Because the drugs are already approved for human use, their pharmacokinetics and safety profiles are known [4-6]. Niclosamide is an FDA approved anthelmintic drug that has primarily been used to treat tapeworm infections over the past 50 years [7]. Intriguingly, niclosamide has recently emerged as a true hit in several screens against various diseases, and it has been identified as a potential anti-cancer agent in various high-throughput screening campaigns. In the treatment of tapeworm infections, uncoupling the mitochondria, inhibiting oxidative phosphorylation, and stimulating adenosine triphosphatase activity have been identified as the mechanisms of niclosamide activity [8, 9]. Moreover, recent studies have found that niclosamide also exhibits anti-cancer activity. In many cancer cells, niclosamide inhibits cell growth and induces apoptosis [10-16]. However, few studies have reported the efficacy of niclosamide in human chondrosarcoma treatment and the mechanisms of niclosamide in the context of chondrosarcoma remain unknown. In this study, we hypothesized that niclosamide could contribute to dysfunctional mitochondrial energy metabolism in CHS cells. Therefore, we examined viability, proliferation, apoptosis, and mito-

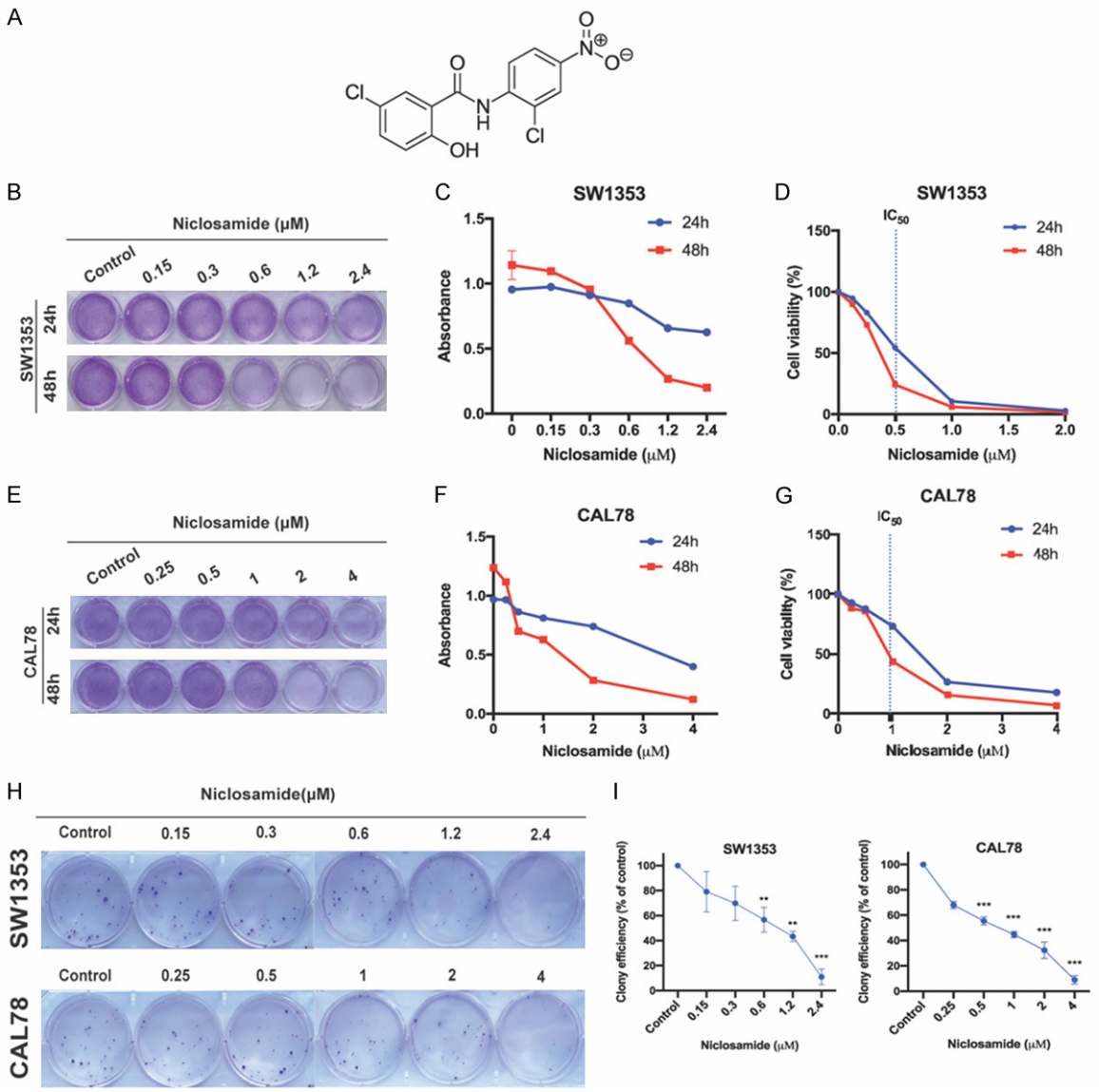

Figure 1. Niclosamide inhibits cell viability and proliferation in SW1353 and CAL78 cells. The schematic diagram displays the chemical molecular structure of niclosamide (A). The crystal violet staining assay (B, E) and corresponding quantitative data (C, F) show that niclosamide significantly inhibits SW1353 and CAL78 cell viability. Niclosamide is highly active and has IC_{50} values of 0.5072 and 0.9472 μM in SW1353 and CAL78 cells, respectively (D, G). The colony forming assay (H) and quantification (I) indicate that niclosamide significantly inhibits cell viability and proliferation in SW1353 and CAL78 cells, in a dose-dependent manner. *P<0.05; **P<0.01; ***P<0.001.

chondrial energy metabolism in CHS cells after niclosamide treatment.

Materials and methods

Cell culture and chemicals

The human CHS cell lines SW1353 and CAL78 were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China) and cultured in DMEM medium (Gibco, Grand Island, NY, USA) that was supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, and 0.1 mg/mL of streptomycin at 37°C in a humidified incubator with 5% CO_2.

Niclosamide, was purchased from Sigma, dissolved in dimethyl sulfoxide (DMSO), and stored at 4°C at a concentration of 5 mM. The chemical molecular structure of niclosamide is presented in **Figure 1A**. Horseradish peroxidase-

conjugated anti-mouse IgG and mouse polyclonal antibodies for caspase-3, caspase-9, and β-tubulin were purchased from Protientech. The Seahorse Cell Mito Stress Test Kit was purchased from Agilent Seahorse Bioscience. All other chemicals were obtained from Beyotime Biotechnology.

Measurement of cell viability with the CCK-8 assay

SW1353 and CAL78 cells were seeded at a density of 10^4 cells per well in 96-well plates for 24 h and subsequently treated with various concentrations of niclosamide for 24 and 48 h. Then, 20 μL of CCK-8 stock solution were added to each well, and the cells were incubated at 37°C for 2 h. The absorbance at 450 nm was measured with a Bio-Rad microplate reader. The cell viability and IC_{50} were calculated from the absorbance values.

Crystal violet staining cell viability assay

SW1353 and CAL78 cells were seeded at a density of 10^5 cells per well in 24-well plates and treated with various concentrations of niclosamide for 24 h and 48 h. At the end of the treatment periods, the cells were washed with PBS, fixed by 4% paraformaldehyde solution and then stained for 30 min at room temperature with a 0.5% crystal violet solution. Images of the cells were captured after washing with tap water and air drying. For the quantification, absorbance values at 570 nm were measured.

Colony forming assay

Cell survival and proliferation were determined with a colony forming assay. SW1353 and CAL78 cells (200 per well) were treated for 12 h with niclosamide at various concentrations in a 6-well plate. Then, the medium was changed to normal growth medium (DMEM with supplements) and the cells were allowed to grow. After 7 days, the cells were stained with crystal violet and images were captured with a digital camera.

Wound healing assay

SW1353 and CAL78 cells were seeded in a 6-well plate. When the cells reached 90% confluence, an artificial wound was introduced by using a P10 pipette tip. The cells were then treated with niclosamide at various concentrations for 24 h. The images of the wounded area were captured at 0 and 24 h with an Olympus microscope (Olympus Corp).

Transwell assay

For the Transwell invasion assay, the upper chambers of 24-well plate Transwell culture inserts (8 μM pore size; Corning Incorporated, Corning, NY, USA) were coated with basement membrane extract (MaxGel ECM, Sigma-Aldrich). The lower chamber compartments were filled with DMEM medium supplemented with 10% FBS. SW1353 and CAL78 cells were harvested, washed, and re-suspended with DMEM medium without FBS. The cells were seeded in the upper chambers in the presence of niclosamide at various concentrations. After incubating for 24 h, the cells in the upper chamber were removed with a swab. The cells that had migrated to the lower layer and adhered to the membrane were stained with crystal violet. The wells were divided into five fields of view and the cells were imaged under a microscope.

Annexin V-FITC flow cytometry assay

SW1353 and CAL78 cells were seeded in 6-well plates for 24 h and treated with or without niclosamide for 24 h. Then, the cells were detached with trypsin, washed with PBS, and re-suspended in Annexin V Binding Buffer at a density of 10^6 cells/mL. The cells were stained with annexin V-FITC for 30 min, followed by counter-staining with propidium iodide (PI) for 15 min at room temperature. After washing, the cells were analyzed by flow cytometry with an ACEA NovoCyte flow cytometer. Data were analyzed with NovoExpress v.1.2.5.

Tunel assay

SW1353 and CAL78 cell apoptosis was detected with the TdT-mediated dUTP nick end labeling (TUNEL) assay. SW1353 and CAL78 cells were incubated with niclosamide at various concentrations for 24 h. The cells were then fixed with 4% paraformaldehyde for 30 min, washed with 0.3% Triton X-100 in PBS for 5 min, and incubated with 0.3% H_2O_2 in PBS for 20 min. Finally, apoptosis was evaluated with the colorimetric TUNEL Apoptosis Assay Kit (Beyotime Biotechnology, Beijing, China) according to the manufacturer's protocol.

Determination of mitochondrial membrane potential

SW1353 and CAL78 cells were seeded in a 6-well plate. After the cells reached 80% confluence, they were treated with various concentrations of niclosamide for 24 h. The JC-1 Mitochondrial Membrane Potential Assay Kit (Beyotime, Beijing, China) was then used for the assay, according to the manufacturer's protocol. The mitochondrial membrane potential (ΔΨm) was determined by flow cytometry analysis with an ACEA NovoCyte flow cytometer. The mitochondria that contained red JC-1 aggregates in the healthy cells were detectable with the PE channel. Green JC-1 monomers in apoptotic cells were detectable with the FITC channel. The intensity ratio of JC-1 aggregates to JC-1 monomers was used to monitor the mitochondria membrane potential change.

Mitochondrial stress assay

The oxygen consumption rate (OCR) in SW1353 and CAL78 cells after niclosamide treatment was measured with the Seahorse XFp Analyzer (Seahorse Bioscience, Agilent). Thirty-six hours prior to starting the assay, SW1353 cells were seeded at a density of 12,000 cells/well. After 6 h, the cells were treated with 1.2 and 2 μM niclosamide for 24 h. The medium was then changed to assay medium (DMEM, pH 7.4) with 5 mM pyruvate and 2 mM glutamine and the cells were incubated for 1 h in a 37°C non-CO_2 incubator. During the assay, 2 μM oligomycin A, 2 μM carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP), and 0.5 μM 1:1 rotenone: antimycin A were sequentially injected to establish the cell metabolic profiles. Data were normalized to the overall cell numbers in each individual well and are presented as the average ± SD of triplicate measurements.

Western blotting analysis

SW1353 and CAL78 cells were treated with various concentrations of niclosamide for 24 h, lysed, collected, and centrifuged at 4°C at 12,000×g for 5 min. The supernatant was collected and protein concentrations were determined with the BCA assay. Equal protein amounts were loaded on 4-20% SDS-PAGE gels using a Mini-PROTEAN Tetra cell system (Bio-Rad, USA). After electrophoresis, the proteins were transferred onto PVDF membranes. The membranes were then incubated with Quick-Block Blocking Buffer (Beyotime Biotechnology), followed by overnight incubation at 4°C with the primary antibodies. The secondary antibodies were incubated for 1 h at room temperature. The following antibodies were used: anti-caspase-3, anti-caspase-9, anti-β-tubulin, and anti-rabbit IgG - horseradish peroxidase-conjugated secondary antibody (1:1,000, Proteintech). The band intensities were analyzed with Image J 2.0 software (NIH, Bethesda, MD, USA). Data are presented as the relative protein levels after normalization to β-tubulin. The ratio of the control samples was assumed to be 1.0.

Statistical analysis

All statistical analyses were performed with GraphPad Prism 8.3.1 software (GraphPad Software, San Diego, California, USA). All data are expressed as the mean ± standard deviation (SD). The Mann-Whitney U test was used for nonparametric analysis of differences between two groups. For comparisons of more than two groups, one-way ANOVA was used. A *P*-value <0.05 was considered statistically significant.

Results

Niclosamide inhibits the growth and proliferation of SW1353 and CAL78 cell lines

To examine the inhibitory effects of niclosamide on the growth and proliferation of SW1353 and CAL78 cell lines, the CCK-8, crystal violet staining, and colony forming assays were performed. The CCK-8 assay results (**Figure 1D**, **1G**) indicated that niclosamide significantly inhibited CHS cell viability. The effect was most pronounced at niclosamide concentrations of 0.5-2 μM and 1-4 μM for SW1353 and CAL78 cells, respectively. Niclosamide at 2 μM was highly active and had IC_{50} values of 0.5072 and 0.9472 μM in SW1353 and CAL78 cells, respectively. Moreover, results from the quantitative crystal violet staining (**Figure 1C**, **1F**) and colony forming assays (**Figure 1I**) suggested that niclosamide could inhibit cell proliferation in SW1353 cells at concentrations of 0.6 μM and higher. Similarly, proliferation in CAL78 cells was inhibited at niclosamide concentrations of 0.5 μM and higher. These results are consistent with the CCK-8 assay results. Collectively,

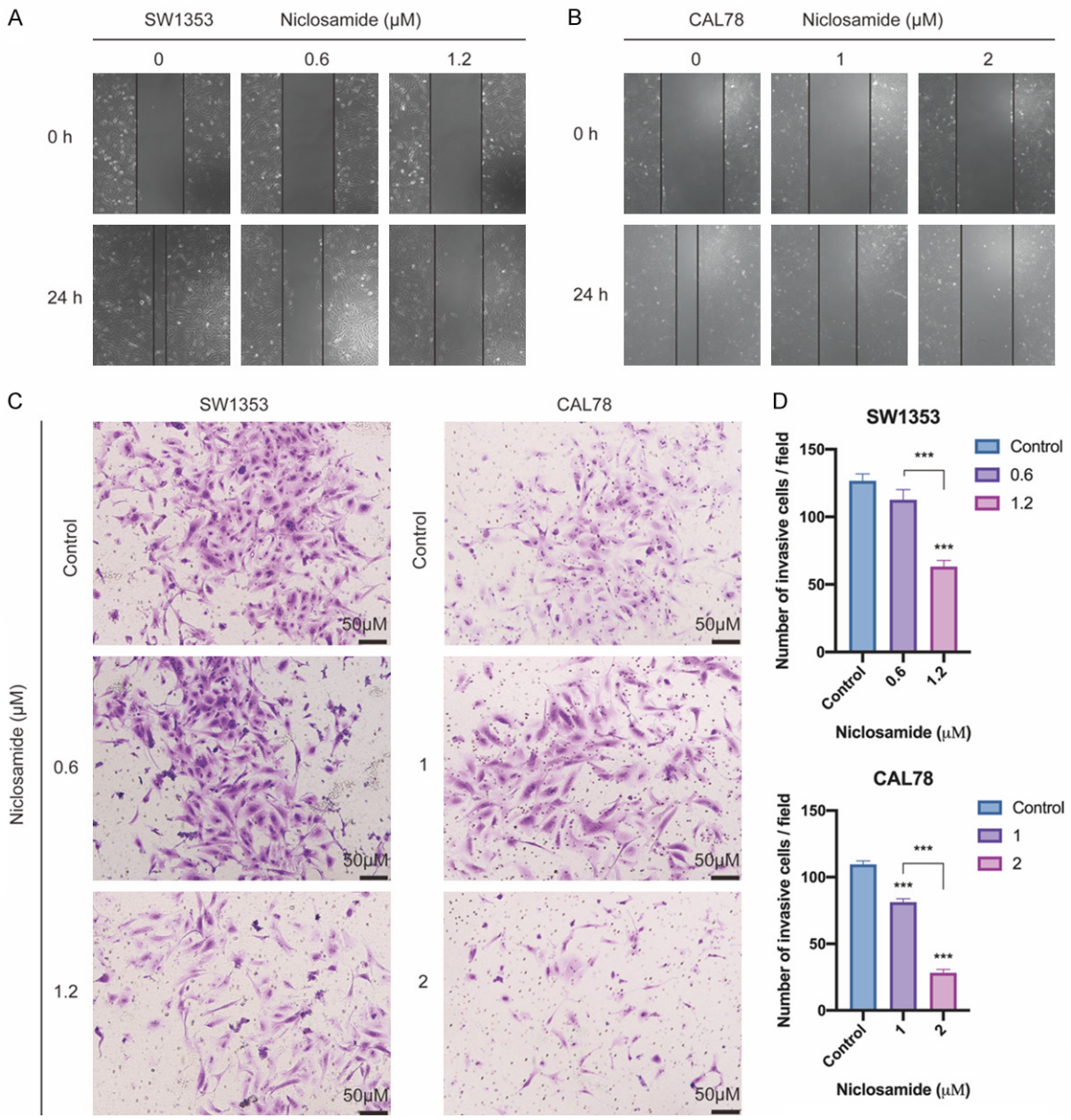

Figure 2. Niclosamide inhibits migratory and invasive behaviors in SW1353 and CAL78 cells. The wound closure in SW1353 and CAL78 cells at 24 h is significantly inhibited after a 24-hour niclosamide treatment at the indicated concentrations (A, B). The number of invasive cells per field decreases after 0.6 and 1.2 μM niclosamide treatments in SW1353 cells. Similar effects are seen in CAL78 cells after treatment with 1 and 2 μM niclosamide (C, D). *P<0.05; **P<0.01; ***P<0.001.

the CCK-8, crystal violet staining, and colony forming assays indicated that cell viability, growth, and proliferation are inhibited by niclosamide.

Niclosamide attenuates the migratory and invasive capacities of SW1353 and CAL78 cell lines

Human chondrosarcoma cells are highly migratory and invasive. We investigated if these cell behaviors could be attenuated by niclosamide, as shown in **Figure 2**. The wound healing assay indicated that niclosamide inhibited SW1353 and CAL78 cell migration. Moreover, similar results were obtained with the Transwell invasion assay. **Figure 2D** displays the number of invasive cells per field, which decreased at niclosamide concentrations of 0.6 and 1 μM. Notably, invasiveness was significantly decreased at niclosamide concentrations of 1.2 and 2 μM in both SW1353 and CAL78 cells. These

results suggested that niclosamide attenuates the migratory and invasive capabilities of SW1353 and CAL78 cells.

Niclosamide induces SW1353 and CAL 78 cell apoptosis

To determine whether niclosamide could induce SW1353 and CAL78 cell apoptosis, cells were incubated with niclosamide and the annexin V-FITC/PI flow cytometry and TUNEL assays were performed. **Figure 3A**, **3B** displays the flow cytometry results from SW1353 and CAL78 cells that were treated with the indicated concentrations of niclosamide and double stained for V-FITC/PI. Niclosamide significantly induced late apoptosis at concentrations of 0.6 and 1.2 μM in SW1353 cells. Both early and late apoptosis were induced in CAL78 cells at niclosamide concentrations of 1 and 2 μM. For the TUNEL assay, **Figure 3C**, the nuclei of living cells were dyed blue with a hematoxylin staining solution and the nuclei of apoptotic cells were dyed brown. The quantitative data in **Figure 3D** demonstrates that the number of apoptotic cells gradually increased with increasing niclosamide concentration, which occurred in a dose-dependent manner. These data indicate that niclosamide induces SW1353 and CAL78 cell apoptosis.

Niclosamide decreases the mitochondrial membrane potential in SW1353 and CAL78 cells

Mitochondria are vital for cellular bioenergetics and they play a central role in determining the apoptotic point-of-no-return. To examine the effect of niclosamide on mitochondria in SW1353 and CAL78 cells, the JC-1 mitochondrial membrane potential assay was performed after cells had been treated with niclosamide for 24 h. As indicated in **Figure 4A**, **4B**, the P2 gate and Q2-2 quadrant represented the healthy cells with mitochondria that contained JC-1 aggregates. Apoptotic cells that contained JC-1 monomers were in the P3 gate and Q2-4 quadrants. The intensity ratio of JC-1 aggregates to JC-1 monomers, which represents the change of mitochondrial membrane potential, decreased with increasing niclosamide concentrations (**Figure 4B**). These data indicate that niclosamide alters the mitochondrial membrane potential in a dose-dependent manner.

Niclosamide inhibits mitochondrial respiration in SW1353 and CAL78 cells

To further evaluate the effect of niclosamide on cellular mitochondrial function, the Seahorse Cell Mito Stress Assay, which evaluates the oxygen consumption rate (OCR), was performed in SW1353 and CAL78 cells after niclosamide treatment. As shown in **Figure 5A**, **5B**, niclosamide treatment altered the cellular responses of typical mitochondrial complex inhibitors including oligomycin A (ATP synthase inhibitor), FCCP, (mitochondrial uncoupler), antimycin A (complex III inhibitor), and rotenone (complex I inhibitor). Together, these data suggest that mitochondrial ATP production, maximal respiration, and spare capacity were inhibited by niclosamide.

Niclosamide induces the activation of caspase-dependent mitochondrial apoptosis

To investigate whether niclosamide induced SW1353 and CAL78 cell apoptosis via the caspase-dependent apoptotic pathway, the expression of caspase-3, cleaved caspase-3, caspase-9, cleaved caspase-9, and β-tubulin levels were investigated by western blotting analysis. As shown in **Figure 6A**, **6B**, cleaved caspase-3 and cleaved caspase-9 levels significantly increased at niclosamide concentrations of 0.6-1.2 μM in SW1353 cells. Cleaved caspase-3 and cleaved caspase-9 levels also significantly increased in CAL78 cells, but at niclosamide concentrations of 1-2 μM. Niclosamide can induce the activation of caspase-dependent mitochondrial apoptosis.

Discussion

As a member of the bone and soft tissue tumor family known as sarcomas, chondrosarcoma is a bone tumor that typically presents in young people at the ends of the long bones [2]. Historically, chondrosarcoma has been characterized by the proliferation of chondrocytes and is accompanied by giant cells and hyaline cartilage differentiation. It is the second most common malignant bone tumor and has a potent capacity to both invade locally and cause distant organic metastasis [3]. Surgical resection is the primary chondrosarcoma treatment. However, clinical outcomes are poor and the chondrosarcoma recurrence rate is 13% [3]. Radiotherapy is not recommended to treat

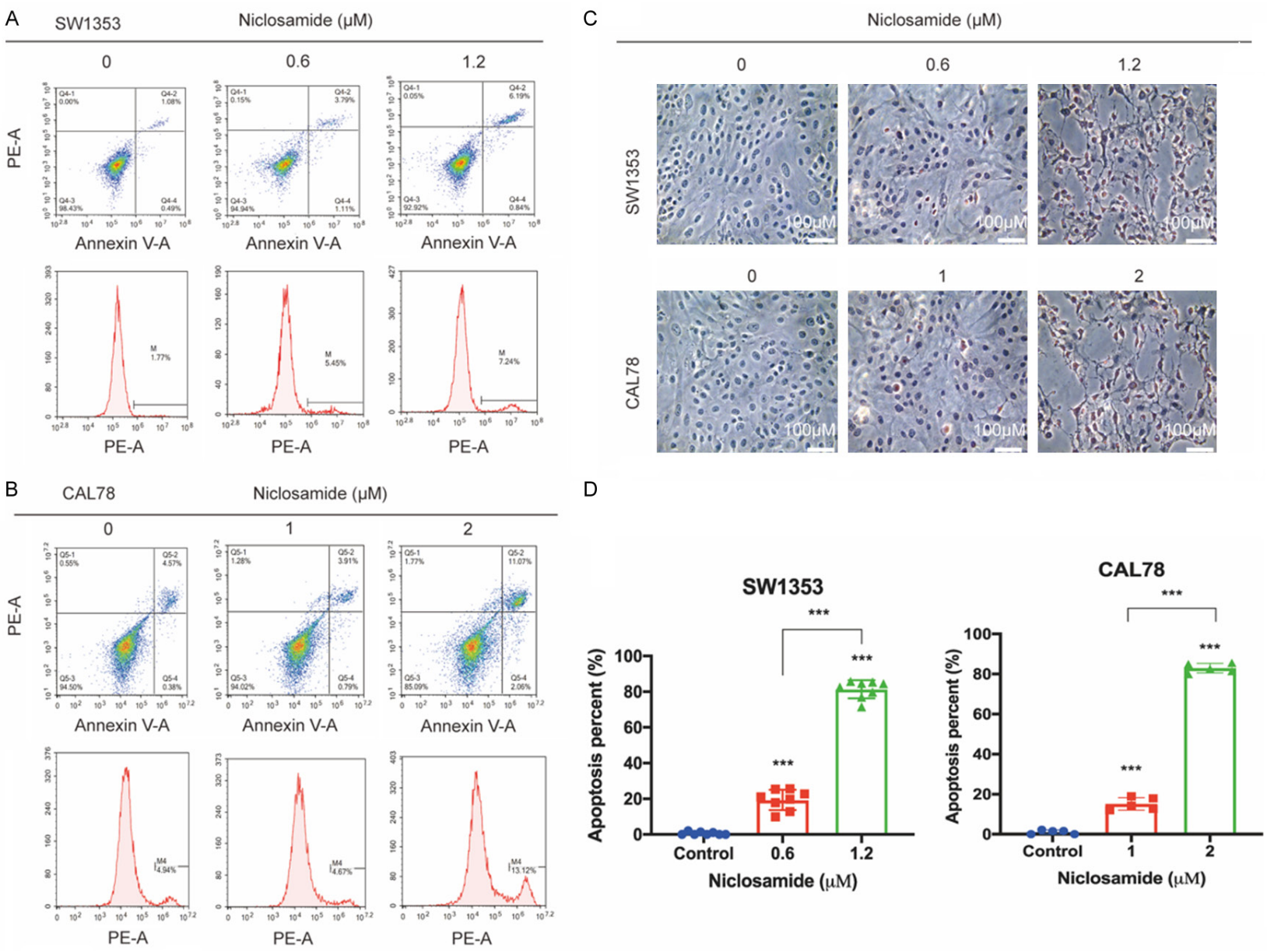
A
SW1353
Niclosamide (μM)
0
0.6
1.2
PE-A
Annexin V-A
PE-A
B
CAL78
Niclosamide (μM)
0
1
2
PE-A
Annexin V-A
PE-A
C
Niclosamide (μM)
0
0.6
1.2
SW1353
100μM
0
1
2
CAL78
100μM
D
SW1353
Apoptosis percent (%)
Control
0.6
1.2
Niclosamide (μM)
CAL78
Control
1
2
Niclosamide (μM)
***

Figure 3. Niclosamide induces apoptosis in SW1353 and CAL78 cells. The annexin V-FITC/PI flow cytometry assay (A, B) shows that niclosamide significantly induces late apoptosis at concentrations of 0.6 and 1.2 μM in SW1353 cells. Both early and late apoptosis are induced at niclosamide concentrations of 1 and 2 μM in CAL78 cells. After hematoxylin staining in the TUNEL assay (C) live cell nuclei are dyed blue and apoptotic cell nuclei are dyed brown. The quantification of data of TUNEL assay (D) indicates that the number of apoptotic cells gradually increased in a dose-dependent manner. *P<0.05; **P<0.01; ***P<0.001.

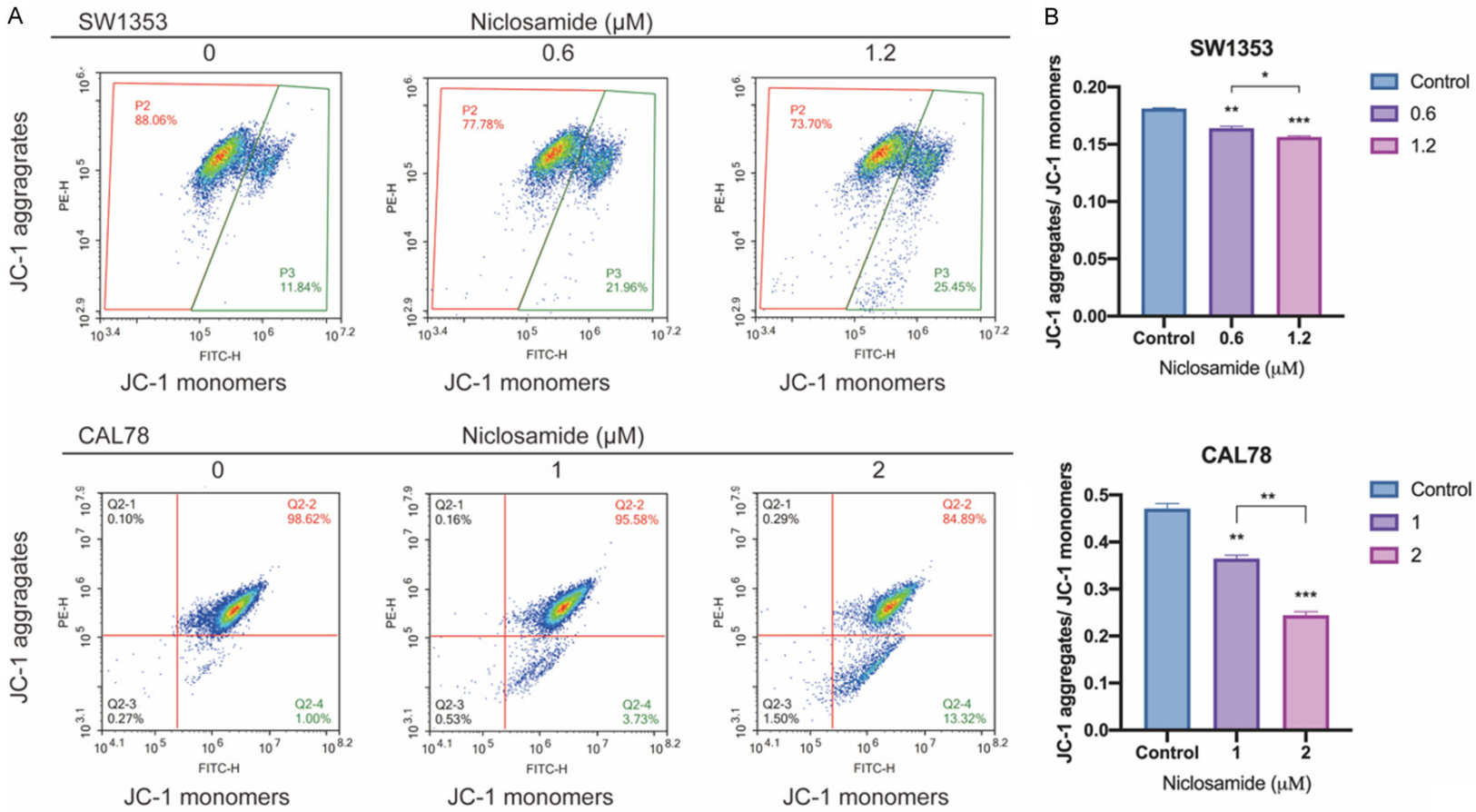

Figure 4. Niclosamide decreases the mitochondrial membrane potential in SW1353 and CAL78 cells. The P2 gate and Q2-2 quadrant represent the healthy cells with mitochondria that contain JC-1 aggregates. Apoptotic cells with JC-1 monomers are in the P3 gate and Q2-4 quadrant (A). The mitochondria membrane potential represented by the intensity ratio of JC-1 aggregates to JC-1 monomers (B) decreases as the concentration of niclosamide increases, which indicates that niclosamide decreases the mitochondrial membrane potential in a dose-dependent manner. *P<0.05; **P<0.01; ***P<0.001.

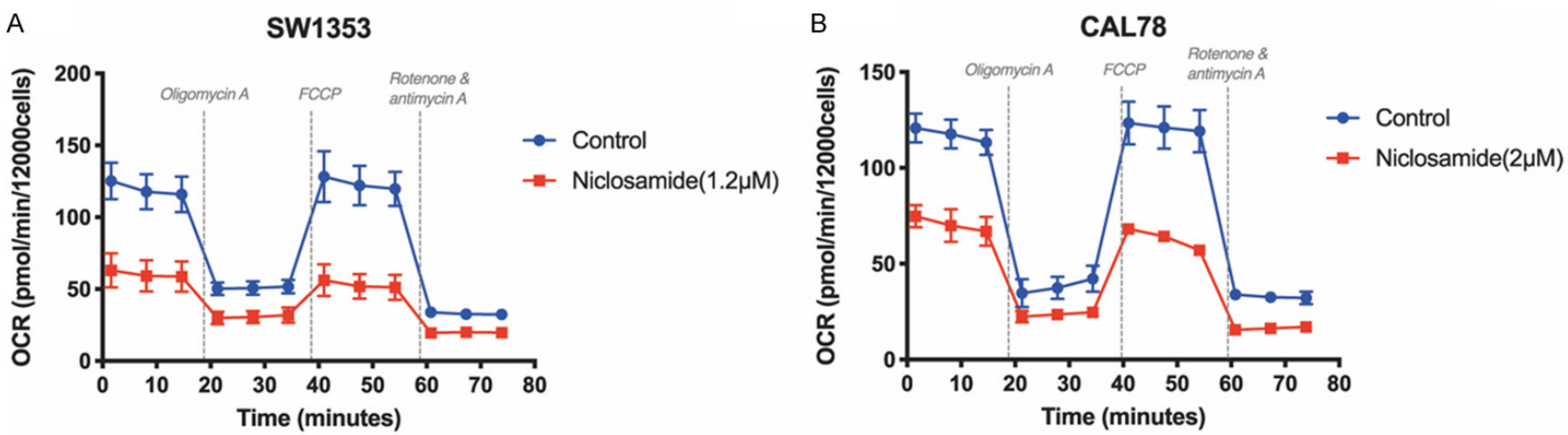

Figure 5. Niclosamide inhibits mitochondrial respiration in SW1353 and CAL78 cells. The oxygen consumption rate (OCR) in SW1353 and CAL78 cells that were treated with 1.2 μM and 2 μM niclosamide for 24 h is measured with the Seahorse Cell Mito Stress assay (A, B). The compounds (oligomycin, FCCP, and a mix of rotenone and antimycin (A) that target components of the electron transport chain (ETC) in the mitochondria are serially injected to measure ATP-linked respiration, maximal respiration, and nonmitochondrial respiration, respectively. The niclosamide treatment alters the cellular responses to typical mitochondrial complexes inhibitors. The mitochondrial ATP production, maximal respiration, and spare capacity are inhibited when compared to the control group.

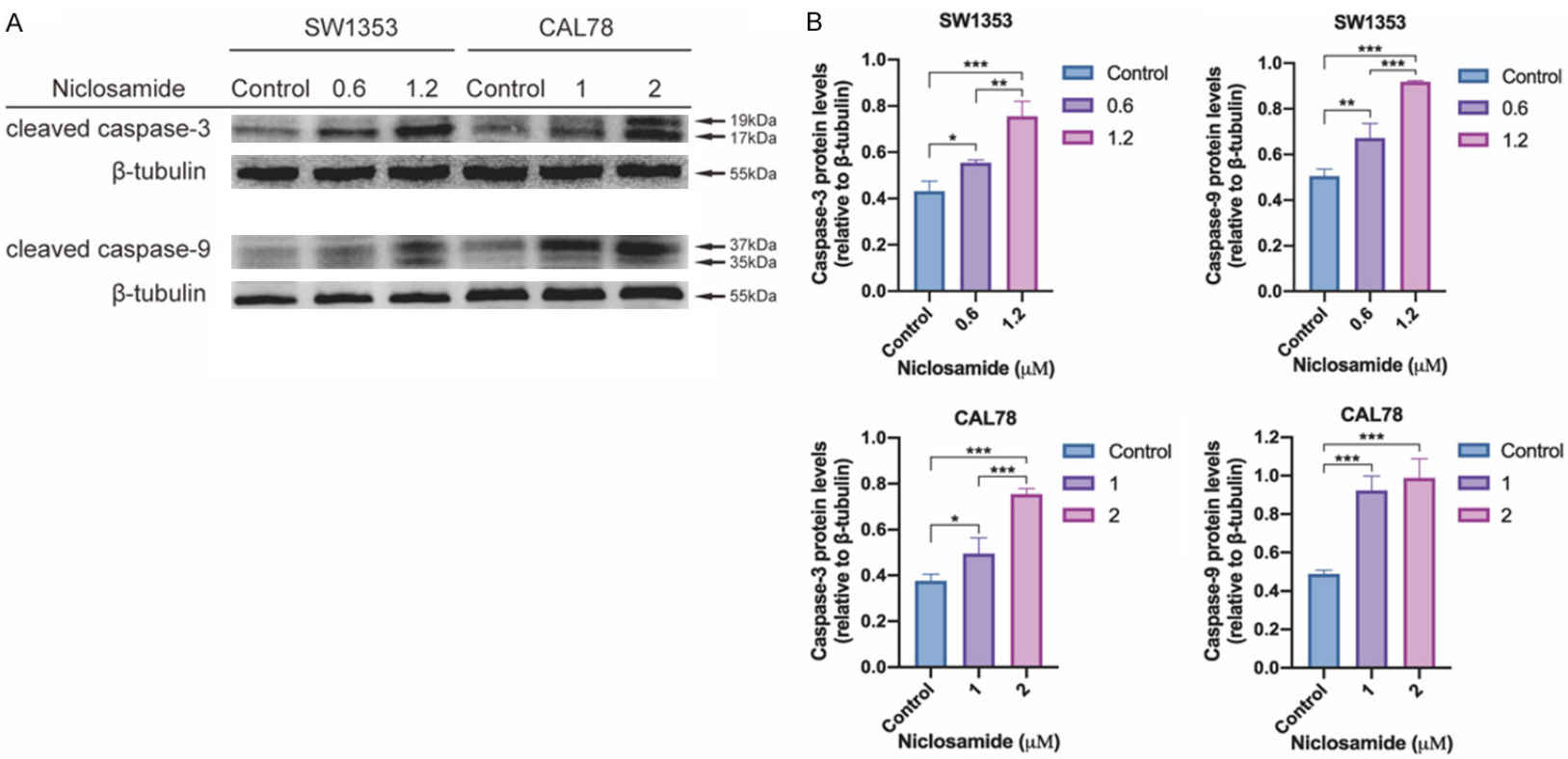

Figure 6. Niclosamide induces the activation of caspase-dependent mitochondrial apoptosis. SW1353 and CAL78 cells were treated with niclosamide at the indicated concentrations for 24 h and the expression levels of cleaved caspase-3, cleaved caspase-9, β-tubulin were investigated with western blotting (A, B). Cleaved caspase-3 and cleaved caspase-9 levels significantly increase at niclosamide concentrations of 0.6-1.2 μM in SW1353 cells and at niclosamide concentrations of 1-2 μM in CAL78 cells, which indicated that niclosamide induces the activation of caspase-dependent mitochondrial apoptosis. *P<0.05; **P<0.01; ***P<0.001.

chondrosarcoma because it has the potential to stimulate malignant progression. The poor sensitivity of this cancer to chemotherapy drugs and high recurrence rate are recognized bottlenecks to successful treatment.

Drug development, starting from the initial discovery of a promising target to finally bringing the drug to market, is an expensive, lengthy, and incremental process [17]. Therefore, using drug repurposing to identify new applications for approved drugs outside the scope of the original indication has been an effective way to accelerate the development of new anti-cancer agents. Importantly, the pharmacokinetics and safety profiles in humans are already known for these drugs [4, 5, 18].

Niclosamide, which is an FDA approved anthelmintic drug [8], has excellent safety profiles in various mammalian species. Niclosamide is also reported to exhibit strong anti-cancer activity against several cancers including colon cancer, breast cancer, osteosarcoma, human glioblastoma, and others [10-12, 14-16, 19-21]. Chen et al. [22] identified that niclosamide is a small molecule inhibitor of Wnt/β-catenin signaling that acts by promoting Wnt receptor endocytosis to downregulate Dvl2 protein in human osteosarcoma cells. In an anti-cancer drug screen, Yi et al. [14] identified that niclosamide had broad-spectrum, anti-cancer activity against 60 human tumor cell lines. The screening results suggested that niclosamide could inhibit cell proliferation of all the tumor cell lines that were tested with the IC_{50} values that were less than 1 μM. Similar niclosamide anti-cancer efficacy against tumor growth and metastasis was shown in xenograft models. Jin et al. [23] first reported the *in vivo* anti-cancer activities of niclosamide in acute myeloid leukemia. The drug inhibited xenograft tumor growth and damaged mitochondria. Moreover, niclosamide also significantly inhibited tumor formation, growth, and metastasis in xenograft models of lung [24] and ovarian cancers [25]. These studies indicate that the *in vitro* and *in vivo* anti-cancer activities of niclosamide are linked to inhibiting cell proliferation, inducing cell apoptosis, damaging mitochondria, and inhibiting multiple signaling pathways.

Similarly, our study showed that niclosamide significantly inhibits cell growth, proliferation, and migratory and invasive behaviors in the human chondrosarcoma SW1353 and CAL78 cell lines. At a safe concentration, niclosamide significantly induced apoptosis of human chondrosarcoma cells. The data collectively demonstrate that niclosamide exhibits anti-cancer activities in human chondrosarcoma cells.

To investigate the impact of niclosamide on mitochondrial function in chondrosarcoma cells, the JC-1 assay was performed. As shown in **Figure 4** niclosamide decreased the mitochondrial membrane potential of chondrosarcoma cells in a dose-dependent manner. The mitochondrial membrane potential is generated by proton pumps (Complexes I, III, and IV) and is an essential component of the energy storage process during oxidative phosphorylation [26]. Mitochondrial uncoupling is a normal physiological process that uncouples ATP production from nutrient oxidation and proton transport [27-30]. Because niclosamide is a mitochondrial uncoupler, it can reduce the mitochondrial membrane potential. Notably, a long-lasting drop in the membrane potential may inhibit cell viability and cause various pathologies including mitochondrial dysfunction. We used the Seahorse Cell Mito Stress Test to examine mitochondrial function. As shown in **Figure 5A**, **5B**, treatment with niclosamide altered the cellular responses to typical mitochondrial complex inhibitors. After oligomycin and FCCP injections, niclosamide-treated cells had a worse response when they were compared to the control group. These data indicate that mitochondrial ATP production, maximal respiration, and spare capacity are inhibited after niclosamide treatment. Alasadi et al. [31] reported that niclosamide had anti-cancer effects on hepatic and colon cancer cells. More specifically, they found that niclosamide, which acts as a mitochondrial uncoupler, exhibited anti-cancer activities that were associated with altered bioenergetics and energy metabolism. In our study, the anti-cancer effect of niclosamide on chondrosarcoma cells could be associated with an increase in the mitochondrial membrane permeability, which reduced the membrane potential, and eventually led to mitochondrial dysfunction. This hypothesis was confirmed by western blotting. Both cleaved caspase-3 and cleaved caspase-9 have key roles in apoptotic signaling pathways and as shown in **Figure 6**, their expression increased after niclosamide treatment.

The cell apoptosis induced by niclosamide in human chondrosarcoma cells is due to dysfunctional mitochondrial energy metabolism. Our current study provides evidence to support this hypothesis: Niclosamide decreased mitochondrial membrane potential and inhibited mitochondrial ATP production, maximal respiration, and spare capacity. Collectively, these changes may trigger the mitochondrial apoptotic pathway. The mitochondria are involved in programmed cell death and specifically, apoptosis. Studies have shown that the permeability transition pore (PTP) and mitochondrial membrane potential have important roles in apoptosis [32-35]. The basic change in mitochondria that promotes apoptosis is altered mitochondrial permeability, which leads to a decreased membrane potential and the release of pro-apoptotic substances. Several studies [36-38] report that there are two main pathways for mitochondria-associated caspase activation: (1) When the mitochondrial membrane potential decreases, the apoptosis-inducing factor (AIF) is released from the mitochondrial extracellular compartment and activates caspase-3; (2) When cytochrome c is released from the mitochondria, it forms a complex with Apaf-1 and caspase-9, which then activates caspase-3. Overall, our study and others demonstrate that niclosamide exhibits potent anti-cancer activity in human chondrosarcoma cells.

Here, we investigated the potential of repurposing niclosamide as an anti-cancer agent for chondrosarcoma. We found that niclosamide significantly inhibited human chondrosarcoma cell growth and induced cell apoptosis at biologically safe doses. The anti-cancer mechanism of niclosamide in chondrosarcoma cells may be related to apoptosis that is induced by disrupted mitochondrial anabolic metabolism. Our study suggests that niclosamide is a potent chondrosarcoma tumor inhibitor that acts via the activation of the caspase-dependent mitochondrial apoptotic pathway. Niclosamide is a novel therapeutic approach to treat chondrosarcoma and provides new clues about the mitochondrial mechanisms of tumor suppression.

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Disclosure of conflict of interest

None.

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