Original Paper

Impact of Na⁺/Ca²⁺ Exchangers on Therapy Resistance of Ovary Carcinoma Cells

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Key Words

NCX3 • NCKX4 • NCKX5 • NCKX6 • Cisplatin • Apoptosis

Abstract

Background/Aims: According to previous observations, enhanced store-operated Ca²⁺-entry (SOCE) accomplished by the pore forming ion channel unit Orai1 and its regulator STIM1 contribute to therapy resistance of ovary carcinoma cells. Ca²⁺ signaling is further shaped by Ca²⁺ extrusion through K⁺-independent (NCX) and/or K⁺-dependent (NCKX) Na⁺/Ca²⁺-exchangers. The present study thus explored whether therapy resistance is further paralleled by altered expression and/or function of Na⁺/Ca²⁺-exchangers. **Methods:** In therapy resistant (A2780cis) and therapy sensitive (A2780sens) ovary carcinoma cells transcript levels were estimated from RT-PCR, cytosolic Ca²⁺-activity ([Ca²⁺]_i) from Fura-2-fluorescence, Na⁺/Ca²⁺-exchanger activity from the increase of [Ca²⁺]_i (Δ[Ca²⁺]_i) and from whole cell current (I_{Ca}) following abrupt replacement of Na⁺ containing (130 mM) and Ca²⁺ free extracellular perfusate by Na⁺ free and Ca²⁺ containing (2 mM) extracellular perfusate, as well as cell death from PI-staining in flow cytometry. **Results:** The transcript levels of NCX3, NCKX4, NCKX5, and NCKX6, slope and peak of Δ[Ca²⁺]_i as well as I_{Ca} were significantly higher in therapy resistant than in therapy sensitive ovary carcinoma cells. The Na⁺/Ca²⁺-exchanger inhibitor KB-R7943 (10 μM) significantly blunted Δ[Ca²⁺]_i and significantly augmented the cisplatin-induced cell death of therapy resistant ovary carcinoma cells without significantly modifying cisplatin-induced cell death of therapy sensitive ovary carcinoma cells. **Conclusion:** Enhanced Na⁺/Ca²⁺-exchanger activity may contribute to the therapy sensitivity of ovary carcinoma cells.

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Introduction

Alterations of cytosolic Ca²⁺ activity contribute to the regulation of diverse cellular mechanisms, such as excitation, exocytosis, migration, cell proliferation and cell death [1-5]. Cytosolic Ca²⁺ concentration ([Ca²⁺]_i) is increased by several mechanisms including Ca²⁺ release from intracellular stores with subsequent triggering of store operated Ca²⁺ entry (SOCE) accomplished by Orai [6-10] and STIM [11-15] isoforms. Orai1 and STIM1 are expressed in and contribute to the survival of several tumor cells [16-20] including therapy resistant ovary carcinoma cells [21].

Cytosolic Ca²⁺ activity is further modified by Ca²⁺ extrusion accomplished by several mechanisms including Na⁺/Ca²⁺ exchangers, which thus contribute to the shaping of duration, amplitude and intracellular location of Ca²⁺ signals [22-24]. Na⁺/Ca²⁺ exchangers operate with far higher turnover rates than Ca²⁺ ATPases [25]. Six K⁺-dependent (NCKX) and three K⁺-independent (NCX) Na⁺/Ca²⁺ exchanger isoforms have been identified [26-28]. NCX isoforms exchange three Na⁺ ions for one Ca²⁺ ion and NCKX isoforms exchange one K⁺ ion and one Ca²⁺ ion for four Na⁺ ions [29]. The direction of transport by Na⁺/Ca²⁺ exchangers depends on the electrochemical driving forces, i.e. the cell membrane potential, the Na⁺ gradient and the Ca²⁺ gradient [30].

The present study explored whether ovary carcinoma cells express Na⁺/Ca²⁺ exchangers and whether Na⁺/Ca²⁺ exchanger expression and function differs between therapy resistant and therapy sensitive ovary carcinoma cells. To this end, the transcript levels of the three NCX and six NCKX isoforms were quantified, Na⁺/Ca²⁺ exchanger activity determined and the impact of NCX inhibition on cisplatin induced cell death analyzed.

Materials and Methods

Ethics Statement

Investigation has been conducted in accordance with the ethical standards and according to the Declaration of Helsinki and according to national and international guidelines and has been approved by the authors' institutional review board.

Cell culture

Experiments were performed in cisplatin-resistant cells (A2780cis) and their therapy sensitive parent cells (A2780sens) (ECACC catalogue no. 93112519). A2780cis has been generated by exposure to increasing concentrations of cisplatin and is further resistant to melphalan, adriamycin and irradiation [31-33]. The cells were cultured in Dulbecco's RPMI media, containing 10% fetal calf serum and 1% antibiotic/antimycotic solution. Where indicated, the cells were treated with cisplatin (100 μM) (Sigma, Taufkirchen, Germany) [21] or the NCX inhibitor [34] KB-R7943 (10 μM) (Sigma, Taufkirchen, Germany).

Real-time PCR

Total RNA was extracted from ovary carcinoma cells in TriFast (Pqlab, Erlangen, Germany) according to the manufacturer's instructions. After DNase digestion reverse transcription of total RNA was performed using Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostics, Penzberg, Germany). Real-time polymerase chain reaction (RT-PCR) of the respective genes were set up in a total volume of 20 μl using 40 ng of cDNA, 500 nM forward and reverse primer and 2x GoTaq® qPCR Master Mix (Promega, Hilden, Germany) according to the manufacturer's protocol. Cycling conditions were as follows: initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 15 sec, 58°C for 15 sec and 68°C for 20 sec. For amplification the following primers were used (5'→3' orientation):

for NCX1: fw: acaagaggtatcgagctggc, rev: atgccatttctgcctagc;
for NCX2: fw: cgctggtgttcaaacag, rev: gacgaccagcagcat;
for NCX3: fw: gcattgccagggtcattgtct, rev: ccataagggtcaggttgaga;
for NCKX1: fw: tccacgcagaagatggtg, rev: gtgatggaggggatagcg;
for NCKX2: fw: gagacagatacacagagcacagg, rev: gagaatagtacagatcacgccc;

for NCKX3: fw: atgacagcagcaactgcga, rev: tcattgatcaacatgcgactgg;

for NCKX4: fw: tcaaagttcgaggaggcg, rev: attcactggggccatcaact;

for NCKX5: fw: ctccatcatcgaggttcc, rev: cttcctaccctccctggaa;

for NCKX6: fw: cgtgctgggtaccacagtgg, rev: cttcgtggcagggtcag;

for GAPDH: fw: tgagtacgtcgtggagtccac, rev: gtgctaagcagttggtgtg.

Specificity of PCR products was confirmed by analysis of a melting curve. Real-time PCR amplifications were performed on a CFX96 Real-Time System (Bio-Rad) and all experiments were done in duplicate. The house-keeping gene GAPDH (Glycerinaldehyd-3-phosphat-Dehydrogenase) was amplified to standardize the amount of sample RNA. Relative quantification of gene expression was achieved using the ΔCT method as described earlier [35, 36].

Ca^{2+} measurements

Fura-2 fluorescence was utilized to determine intracellular Ca^{2+} activity [37]. Cells were loaded with Fura-2/AM (2 μM , Invitrogen, Goettingen, Germany) for 20–60 min at 37°C. Cells were excited alternatively at 340 nm and 380 nm through an objective (Fluor 40 $\times$ /1.30 oil) built in a fluorescence microscope (Axiovert 100, Zeiss, Oberkochen, Germany). Emitted fluorescence intensity was recorded at 505 nm. Data were acquired using specialized computer software (Metafluor, Universal Imaging, Downingtown, USA).

$\text{Na}^+/\text{Ca}^{2+}$ exchanger activity was estimated from the changes in cytosolic Ca^{2+} activity upon replacement of extracellular Na^+ by N-methyl-D-glucamine (NMDG). The standard Na^+ containing solution was composed of (in mM): 130 NaCl, 0 KCl, 2 MgCl_2 , 10 HEPES, 5 glucose, pH 7.4 and the Na^+ -free solution of (in mM): 90 NMDG, 0 KCl, 2 CaCl_2 , 2 MgCl_2 , 10 HEPES, 5 glucose, pH 7.4. For determination of NCKX activity the Na^+ containing solution was composed of (in mM): 130 NaCl, 40 KCl, 20 TAE $^+$, 2 MgSO_4 , 10 HEPES, 5 glucose, pH 7.4 and the Na^+ -free solution of (in mM): 90 NMDG, 40 KCl, 20 TAE $^+$, 2 CaCl_2 , 2 MgCl_2 , 10 HEPES, 5 glucose, pH 7.4. For quantification of Ca^{2+} entry, the slope (delta ratio/s) and peak (delta ratio) were calculated following removal of Na^+ .

Patch clamp

Patch clamp experiments were performed at room temperature in voltage-clamp, fast-whole-cell mode according to Hamill et al. [38]. The cells were continuously superfused through a flow system inserted into the dish. Borosilicate glass pipettes (2–5 M Ω tip resistance; Harvard Apparatus, Kent, UK) manufactured by a microprocessor-driven DMZ puller (Zeitz, Augsburg, Germany) were used in combination with a MS314 electrical micromanipulator (MW, Märzhäuser, Wetzlar, Germany). The currents were recorded by an EPC-9 amplifier (Heka, Lambrecht, Germany) using Pulse software (Heka) and an ITC-16 Interface (Instrutech, Port Washington, N.Y., USA). The currents were recorded with an acquisition frequency of 10 kHz and 3 kHz low-pass filtered. The offset potentials between both electrodes were zeroed before sealing. Whole-cell currents elicited by changing the bath solutions were measured during a continuous 40 second square wave voltage pulse to -80 mV. The applied voltages refer to the cytoplasmic face of the membrane with respect to the extracellular space. The outward currents, defined as flow of positive charge from the cytoplasmic to the extracellular membrane face, are positive currents and depicted as outward deflections of the original current traces. To measure $\text{Na}^+/\text{Ca}^{2+}$ exchanger-mediated currents, a Na^+ -based pipette solution was used (in mM): 120 NaCl, 40 KCl, 20 TEA-Cl, 2 MgCl_2 , 2 Mg-ATP, 10 HEPES (pH 7.2/CsOH) and 1 μM free Ca^{2+} . The external first solution contained (in mM): 130 NaCl, 20 TEA-Cl, 2 MgCl_2 , 5 glucose, 10 HEPES, 0.5 EGTA (pH 7.5/CsOH). $\text{Na}^+/\text{Ca}^{2+}$ exchange currents were elicited by switching from the first bath solution to a bath solution that contained (in mM): 130 NMDG-Cl, 20 TEA-Cl, 2 MgCl_2 , 2 CaCl_2 , 5 glucose and 10 HEPES (pH 7.5/CsOH). The KCl content of the bath solutions was either 0 or 40 mM.

Determination of apoptosis

To determine apoptosis, 10 5 cells/100 μl in complete RPMI were centrifuged at 1000 rpm for 5 minutes at 24°C, added to 200 μl of PBS containing 50 $\mu\text{g}/\text{ml}$ propidium iodide (Mabtag, Germany) as well as incubated at room temperature in the dark for 10 minutes. The cells were washed once at 1000 rpm for 5 minutes, resuspended in PBS and measured immediately with an excitation wavelength of 488 nm and an emission wavelength of 585 nm (FL-2) with flow cytometry [39] utilizing a FACS Calibur (BD, Heidelberg, Germany).

Statistical analysis

Data are provided as means $\pm$ SEM; n represents the number of independent experiments. All data were tested for significance using Student's unpaired two-tailed t -test, one sample t -test or ANOVA (Dunnett's test) where applicable. Results with $p < 0.05$ were considered statistically significant.

Results

The present study addressed the putative role of $\text{Na}^+/\text{Ca}^{2+}$ exchangers in therapy resistance of ovary carcinoma cells. We first quantified and compared the different transcript levels of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger isoforms in therapy sensitive A2780sens and therapy resistant A2780cis ovary carcinoma cells using quantitative real-time-PCR. To this end the respective transcript levels were quantified in therapy sensitive A2780sens and the therapy resistant A2780cis ovary carcinoma cells by using real time-PCR. As illustrated in Fig. 1, both cell lines express the NCX isoforms NCX1 and NCX3, but very little NCX2 (Fig. 1A). The transcript levels of NCX3, but not those of NCX1 were significantly higher in therapy resistant than in therapy sensitive ovary carcinoma cells (Fig. 1B). As shown in Fig. 2, ovary carcinoma cells further express several NCKX isoforms. The transcript levels of NCKX1 were similar in therapy resistant and in therapy sensitive ovary carcinoma cells. However, the transcript levels of NCKX4, NCKX5, and NCKX6 were significantly higher in therapy resistant as compared to therapy sensitive ovary carcinoma cells (Fig. 2B). NCKX3 transcript levels were not detectable (data not shown).

In the next step, Fura-2 fluorescence was employed to explore whether the differences in NCX and NCKX transcript levels were paralleled by the respective differences in $\text{Na}^+/\text{Ca}^{2+}$ exchanger activity. Therefore the $\text{Na}^+/\text{Ca}^{2+}$ -exchanger activity was estimated from the increase of cytosolic Ca^{2+} activity ($\Delta[\text{Ca}^{2+}]_i$) after the abrupt replacement of a Na^+ -based

Fig. 1. NCX isoform expression in therapy sensitive and therapy resistant ovary carcinoma cells. A. Original blots of NCX isoform transcripts in therapy sensitive (a) and therapy resistant (b) ovary carcinoma cells. For comparison, a size marker for NCX1 (195bp), NCX2 (223bp), NCX3 (201bp) and GAPDH (200bp) is shown. B. Arithmetic means ($\pm$ SEM, $n = 7$ preparations) of NCX1 (a) and NCX3 (b) over GAPDH transcript levels in therapy sensitive (white bars) and therapy resistant (black bars) ovary carcinoma cells. ** ($p < 0.01$) indicates statistically significant difference from therapy sensitive ovary carcinoma cells (student's t -test).

Fig. 2. NCKX isoforms expression in therapy sensitive and therapy resistant ovary carcinoma cells. A. Original blots of NCKX isoform transcripts in therapy sensitive (a) and therapy resistant (b) ovary carcinoma cells. For comparison, a size marker for NCKX1 (138bp), NCKX2 (169bp), NCKX3 (205bp), NCKX4 (179bp), NCKX5 (143bp), NCKX6 (144bp), and GAPDH (200bp) is shown. B. Arithmetic means ($\pm$ SEM, $n = 5$ preparations) of NCKX1(a) and NCKX2 (b), NCKX4 (c), NCKX5 (d), NCKX6 (e) over GAPDH transcript levels in therapy sensitive (white bars) and therapy resistant (black bars) ovary carcinoma cells. ** ($p < 0.01$), *** ($p < 0.001$) indicate statistically significant difference from therapy sensitive ovary carcinoma cells (student's t-test).

(Na^+ 130 mM) and Ca^{2+} -free extracellular perfusate by a Na^+ -free and Ca^{2+} -containing (2 mM) extracellular solution. Prior to Na^+ removal the Fura2-fluorescence ratio was similar in therapy sensitive (0.53 ± 0.01 a.u., $n = 8$) and therapy resistant (0.56 ± 0.01 a.u., $n = 10$) ovary carcinoma cells.

As illustrated in Fig. 3, the removal of external Na^+ and addition of external Ca^{2+} was followed by an increase of $[\text{Ca}^{2+}]_i$, which was significantly more pronounced in therapy resistant than in therapy sensitive ovary carcinoma cells. Fig. 3 further illustrates that the increase of cytosolic Ca^{2+} activity following removal of external Na^+ and addition of external Ca^{2+} was virtually abrogated by the $\text{Na}^+/\text{Ca}^{2+}$ exchanger inhibitor KB-R7943 (10 μM).

Further experiments explored whether K^+ -dependent $\text{Na}^+/\text{Ca}^{2+}$ -exchanger activity (NCKX) was different in therapy sensitive and resistant ovary carcinoma cells. To this end, experiments were performed in the presence of high (40 mM) extracellular K^+ concentration. Again, the increase of cytosolic Ca^{2+} activity ($\Delta[\text{Ca}^{2+}]_i$) following abrupt replacement of Na^+ containing (130 mM) and Ca^{2+} free extracellular perfusate by Na^+ free and Ca^{2+} containing (2 mM) extracellular perfusate was taken as evidence for $\text{Na}^+/\text{Ca}^{2+}$ -exchanger activity. As illustrated in Fig. 4, in the presence K^+ , the removal of external Na^+ and addition of external Ca^{2+} was followed by an increase of $[\text{Ca}^{2+}]_i$, which was significantly more pronounced in therapy resistant than in therapy sensitive ovary carcinoma cells. Fig. 4 further illustrates

Fig. 3. NCX mediated Ca^{2+} entry in therapy sensitive and therapy resistant ovary carcinoma cells. A. Representative original tracings showing intracellular Ca^{2+} concentrations in Fura-2/AM loaded therapy sensitive (open symbols) and therapy resistant (closed symbols) ovary carcinoma cells in absence (circles) or presence (triangles) of the NCX inhibitor KB-R7943 (10 μM) prior to and following removal of external Na^+ (0 Na^+) and adding 5 mM K^+ . B,C. Arithmetic means ($\pm$ SEM, $n = 109$ -156- cells from 4-6 group) of the peak (B) and slope (C) of the change in intracellular Ca^{2+} concentrations in therapy sensitive (white bars) and therapy resistant (black bars) ovary carcinoma cells prior to and following removal of external Na^+ (0 Na^+) and adding 5 mM K^+ in the absence (left bars, -KBR) and presence (right bars, + KBR) of the NCX inhibitor KB-R7943 (10 μM). ** ($p < 0.01$), *** ($p < 0.001$) indicate statistically significant difference from therapy sensitive ovary carcinoma cells; \$\$ ($p < 0.01$), \$\$\$ ($p < 0.001$) indicates statistical significant difference from absence of KB-R7943 in the therapy resistant ovary carcinoma cells; \$\$\$\$ ($p < 0.001$) indicates statistical significant difference from the absence of KB-R7943 in the sensitive ovary carcinoma cells (ANOVA).

that the increase of cytosolic Ca^{2+} activity following removal of external Na^+ and addition of external Ca^{2+} was virtually abrogated by NCX inhibitor KB-R7943 (10 μM).

In order to further examine the functionality and membrane insertion of the $\text{Na}^+/\text{Ca}^{2+}$ ex-changers, whole cell currents were measured utilizing the patch clamp technique. Again, the current was recorded following abrupt replacement of Na^+ -containing / Ca^{2+} -free extracellular perfusate by Na^+ -free/ Ca^{2+} -containing (2 mM) solution. The removal of external Na^+ and addition of Ca^{2+} was followed by an outward current, which was significantly larger in therapy resistant than in therapy sensitive ovary carcinoma cells (Fig. 5).

A further series of experiments addressed the impact of the $\text{Na}^+/\text{Ca}^{2+}$ exchangers on the therapy resistance of ovary carcinoma cells. To this end, therapy resistant and therapy sensitive ovary carcinoma cells were treated with cisplatin (100 μM , 24 h) in the absence or presence of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger inhibitor KB-R7943 (10 μM). Cisplatin treatment significantly increased the percentage of propidium iodide containing ovary carcinoma cells, an effect significantly more pronounced in therapy sensitive than in therapy resistant ovary carcinoma cells (Fig. 6). The additional treatment with KB-R7943 did not significantly affect cell death of therapy sensitive ovary carcinoma cells; but significantly increased the percentage of apoptotic therapy resistant ovary carcinoma cells (Fig. 6C).

Fig. 4. NCKX mediated Ca^{2+} entry in therapy sensitive and therapy resistant ovary carcinoma cells. A. Representative original tracings showing intracellular Ca^{2+} concentrations in Fura-2/AM loaded therapy sensitive (open symbols) and therapy resistant (closed symbols) ovary carcinoma cells in absence (circles) or presence (triangles) of the NCX inhibitor KB-R7943 (10 μM) before and following removal of external Na^+ (0 Na^+), addition of 40 mM K^+ and application of K^+ channel blocker TEA $^+$. B,C. Arithmetic means ($\pm$ SEM, $n = 133$ -179- cells from 4-5 group) of the peak (B) and slope (C) of the change in intracellular Ca^{2+} concentrations in therapy sensitive (white bars) and therapy resistant (black bars) ovary carcinoma cells prior to and following removal of external Na^+ (0 Na^+) in the presence of 40 mM K^+ in the absence (left bars, -KBR) and presence (right bars, + KBR) of the NCX inhibitor KB-R7943 (10 μM). *** ($p < 0.001$) indicate statistically significant difference from therapy sensitive ovary carcinoma cells; \$\$\$ ($p < 0.001$) indicates statistical significant difference from the absence of KB-R7943 in the therapy resistant ovary carcinoma cells; § ($p < 0.05$) indicates statistical significant difference from the absence of KB-R7943 in the sensitive ovary carcinoma cells (ANOVA).

Discussion

The present study revealed the expression of several $\text{Na}^+/\text{Ca}^{2+}$ exchanger isoforms in ovary carcinoma cells. In both, therapy resistant and therapy sensitive ovary carcinoma cells, transcript levels were detected encoding the $\text{Na}^+/\text{Ca}^{2+}$ exchangers NCX1, NCX3, NCKX1, NCKX2, NCKX4, NCKX5, and NCKX6. More importantly, the transcript levels of NCX3 and of NCKX4, NCKX5 and NCKX6 were more abundant in therapy resistant than in therapy sensitive ovary carcinoma cells. The increased abundance of mRNA encoding the respective $\text{Na}^+/\text{Ca}^{2+}$ exchanger isoforms was paralleled by similar increases of $\text{Na}^+/\text{Ca}^{2+}$ exchanger activities.

Previous observations disclosed increased expression of both, Orai1 and STIM1 in therapy resistant as compared to therapy sensitive ovary carcinoma cells [21]. The increased expression of Orai1 and STIM1 was paralleled by increased store operated Ca^{2+} entry (SOCE) in therapy resistant ovary carcinoma cells. Pharmacological inhibition of Orai1 augmented cisplatin induced apoptosis of therapy resistant ovary carcinoma cells and virtually abrogated the differences in cisplatin sensitivity between therapy sensitive and therapy resistant ovary carcinoma cells [21]. The Orai isoforms Orai1, 2, or 3 [6-9] and their regulators STIM 1 or 2

Fig. 5. $\text{Na}^+/\text{Ca}^{2+}$ exchanger currents in therapy sensitive and therapy resistant ovary carcinoma cells. A,B. Original tracings of whole cell currents in therapy sensitive (A) and therapy resistant (B) ovary carcinoma cells recorded at -80 mV during the switch between external solutions that contained 40 mM K^+ and either 130 mM Na^+ and no Ca^{2+} or 2 mM Ca^{2+} and no Na^+ . The internal solution stimulated Na^+ - and Ca^{2+} overload (1 μM free Ca^{2+} , 120 mM Na^+ , 40 mM K^+). Cesium and TEA⁺ were present in the solutions to block K^+ channel currents. C. Arithmetic means ($\pm$ SEM, $n = 5-9$ cells) current density changes (ΔI , pA/pF) at -80 mV in therapy sensitive (white bars) and therapy resistant (black bars) ovary carcinoma cells induced by the switch between external solutions containing (in mM) 40 K^+ and 130 Na^+ , 0 Ca^{2+} or 0 Na^+ , 2 Ca^{2+} . ** ($p < 0.01$) indicates statistically significant difference from therapy sensitive ovary carcinoma cells (student's t-test).

[12, 13, 15] have been implicated in survival, proliferation, and migration of tumor cells [16-20, 40-48].

At first glance, it appears counterintuitive that both enhanced Ca^{2+} entry and accelerated Ca^{2+} extrusion confer resistance to apoptosis. However, the concerted action of SOCE and Ca^{2+} extrusion mechanisms such as $\text{Na}^+/\text{Ca}^{2+}$ exchangers lead to the appearance of Ca^{2+} oscillations [49] which are decisive in the regulation of diverse cellular functions [23, 50-53], such as entrance into the S and the M phase of the cell cycle [54, 55] and support of tumor cell survival [56, 57]. Those Ca^{2+} oscillations are equally dependent on timely entry and timely extrusion of Ca^{2+} . Failure to extrude Ca^{2+} leads to sustained increase of cytosolic Ca^{2+} activity, which is a well-known trigger of apoptosis [51, 53, 58-66]. Accordingly, fine tuning of both, Ca^{2+} entry and Ca^{2+} extrusion is required in order to establish the adequate Ca^{2+} oscillations.

In theory, other NCX or NCKX isoforms may be relevant for survival and therapy resistance of other tumor cells. Similarly, other Orai/STIM isoforms [67-69] or other Ca^{2+} channels [70-75] may confer survival and thus establish therapy resistance of other tumor cell types. Disruption of Ca^{2+} oscillations by pharmacological inhibition of the respective channels, of the respective $\text{Na}^+/\text{Ca}^{2+}$ exchanger isoforms and/or the respective regulating kinases may enhance sensitivity to cytostatic therapy or radiation.

In conclusion, ovary carcinoma cells express several $\text{Na}^+/\text{Ca}^{2+}$ exchanger isoforms. The expression of several of those isoforms and thus $\text{Na}^+/\text{Ca}^{2+}$ exchanger activity is higher in therapy resistant than in therapy sensitive ovary carcinoma cells and pharmacological inhibition of $\text{Na}^+/\text{Ca}^{2+}$ exchange sensitises the therapy resistant ovary carcinoma cells to cisplatin. Accordingly, $\text{Na}^+/\text{Ca}^{2+}$ exchangers may be novel targets in the attempt to overcome therapy resistance of tumor cells.

Fig. 6. Effect of NCX inhibitor KB-R7943 on cisplatin induced cell death of therapy sensitive and therapy resistant ovary carcinoma cells. A,B. Original histograms of propidium iodide abundance in therapy sensitive (A) and therapy resistant (B) ovary carcinoma cells prior to (grey areas) and following cisplatin treatment in the absence (black line) and presence (red line) of NCX inhibitor KB-R7943 (10 μM). C. Arithmetic means ($\pm$ SEM, $n = 6-8$) of the percentage of therapy sensitive (white bars) and therapy resistant (black bars) ovary carcinoma cells containing propidium iodide prior to (control, left bars) and following (cisplatin, middle and right bars) treatment with cisplatin (100 μM , 24 h) in the absence (middle bars, - KBR) or presence (right bars, + KBR) of NCX inhibitor KB-R7943 (10 μM). ** ($p < 0.01$), indicate statistically significant difference from the absence of KB-R7943 (10 μM) in cisplatin treated resistant ovary carcinoma cells (student's t-test).

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Disclosure Statement

The authors of this manuscript state that they have no conflicts of interest to declare.

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