Sorcin Induces a Drug-Resistant Phenotype in Human Colorectal Cancer by Modulating Ca\textsuperscript{2+} Homeostasis

Francesca Maddalena\textsuperscript{1}, Gabriella Laudiero\textsuperscript{3}, Annamaria Piscazzi\textsuperscript{1}, Agnese Secondo\textsuperscript{4}, Antonella Scorziello\textsuperscript{4}, Valentina Lombardi\textsuperscript{1}, Danilo Swann Matassa\textsuperscript{3}, Alberto Fersini\textsuperscript{2}, Vincenzo Neri\textsuperscript{2}, Franca Esposito\textsuperscript{3}, and Matteo Landriscina\textsuperscript{1,5}

Medical Biotechnology and Neuroscience, University of Naples Federico II, Naples; Departments of Biochemistry and II, Naples; and IRCCS CROB, Rionero in Vulture, Italy

Abstract

The Ca\textsuperscript{2+}-binding protein sorcin regulates intracellular calcium homeostasis and plays a role in the induction of drug resistance in human cancers. Recently, an 18 kDa mitochondrial isoform of sorcin was reported to participate in antiapoptosis in human colorectal cancer (CRC), but information remains lacking about the functional role of the more abundant 22 kDa isoform of sorcin expressed in CRC. We found the 22 kDa isoform to be widely expressed in human CRC cells, whether or not they were drug resistant. Its upregulation in drug-sensitive cells induced resistance to 5-fluorouracil, oxaliplatin, and irinotecan, whereas its downregulation sensitized CRC cells to these chemotherapeutic agents. Sorcin enhances the accumulation of Ca\textsuperscript{2+} in the endoplasmic reticulum (ER), preventing ER stress, and, in support of this function, we found that the 22 kDa isoform of sorcin was upregulated under conditions of ER stress. In contrast, RNAi-mediated silencing of sorcin activated caspase-3, caspase-12, and GRP78/BiP, triggering apoptosis through the mitochondrial pathway. Our findings establish that CRC cells overexpress sorcin as an adaptive mechanism to prevent ER stress and escape apoptosis triggered by chemotherapeutic agents, prompting its further investigation as a novel molecular target to overcome MDR. Cancer Res; 71(24): 7659–69. ©2011 AACR.

Introduction

Several mechanisms are responsible for inducing both drug resistance in human cancer cells and, among other functions, the upregulation of antiapoptotic genes (1). The investigation of these survival mechanisms responsible for drug resistance is a critical issue, because the likelihood of designing novel molecular targeted strategies, which would rescue the sensitivity of tumor cells to anticancer agents, relies strongly on such knowledge. This issue is extremely relevant in the clinical management of human colorectal cancer (CRC), the second most common cause of cancer mortality (2, 3). Of note, although systemic therapy for metastatic CRC has been significantly improved, the outcome for this malignancy remains poor, with a median overall survival rate ranging from 18 to 24 months (4). Thus, novel molecular targeted strategies are urgently needed to revert drug resistance and improve the efficacy of systemic therapy in human CRC.

We have previously reported that TRAP1, a mitochondrial chaperone with antioxidant and antiapoptotic functions (5–7), is involved in MDR in human CRC cells (8). Indeed, TRAP1 is upregulated in the majority of human CRCs (8), as well as in other cancer types (9, 10), and its upregulation results in a phenotype resistant to multiple chemotherapeutics in CRC cells (8). Accordingly, the downregulation of TRAP1 by siRNA resensitizes tumor cells to apoptotic stimuli (8, 11). Starting from a proteomic analysis of TRAP1 coinmunoprecipitation complexes, we observed that TRAP1 specifically interacts with the 18-kDa mitochondrial isoform of sorcin in a Ca\textsuperscript{2+}-dependent manner and that this interaction is required for the antiapoptotic function of TRAP1. Interestingly, despite the significant homology between the 18 and the 22 kDa isoforms of sorcin, the 22 kDa isoform is not a TRAP1-interacting protein (11).

Sorcin is a Ca\textsuperscript{2+}-binding protein and is a member of the penta-EF-hand protein family (12). Sorcin is a Ca\textsuperscript{2+} sensor which regulates the activity of the ryanodine receptor RyRs, the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger NCX, and the voltage-dependent L-type Ca\textsuperscript{2+} channel (12–14) and that, by the interaction with these target proteins, is involved both in regulating Ca\textsuperscript{2+} homeostasis and in modulating excitation–contraction coupling in the heart (15, 16). Some evidence suggests involvement of sorcin in the drug resistance shown by human malignancies. Indeed, sorcin was purified from a vincristine-resistant lymphoma cell line (17), whereas its overexpression has also been associated with resistance to vincristine in gastric cancer cells (18),...
cancer cells resistant to single agents were selected as previously reported (11). Specific proteins were detected by using rabbit polyclonal anti-sorcin (Ab57991; Abcam), rabbit polyclonal anti-sorcin (a kind gift from Prof. E. Chiancone, University of Rome "La Sapienza"), mouse monoclonal anti-GAPDH (glyceraldehyde-3-phosphate dehydrogenase; sc-47724; Santa Cruz Biotechnology), mouse monoclonal anti-tubulin (sc-8035; Santa Cruz Biotechnology), mouse monoclonal anti-TRAP1 (sc-13557; Santa Cruz Biotechnology), rabbit polyclonal anti-Calnexin (M-108, sc-5627; Santa Cruz Biotechnology), rabbit polyclonal anti–Caspase-12 full length (SPA-827; StressGen), rabbit polyclonal anti–Caspase-12 cleaved form (Ab62463; Abcam), mouse monoclonal anti–Caspase-3 (sc-56051; Santa Cruz Biotechnology), mouse monoclonal anti-COX IV antibodies (MS407; Mitosciences).

RNA extraction and real-time RT-PCR analysis
Total RNA from cell pellets and tumor specimens was extracted using the TRIzol Reagent (Invitrogen). Primers and experimental conditions are reported as Supplementary Methods.

\[ [\text{Ca}^{2+}]_i \] and \([\text{Ca}^{2+}]_m \) measurement and mitochondrial membrane potential

\([\text{Ca}^{2+}]_i \) was measured by single-cell computer-assisted video imaging (29). The equation of Grynkiewicz and colleagues was used for calibration (30). \( \text{Ca}^{2+} \) content into ER was evaluated by using the irreversible and selective inhibitor of the sarco(endo)plasmic reticulum \( \text{Ca}^{2+} \)-ATPase (SERCA) thapsigargin (Tg; 1 mmol/L; ref. 31). \([\text{Ca}^{2+}]_m \) was assessed using the fluorescent dye X-RhoD1 (32). Mitochondrial membrane potential was assessed using the fluorescent dye tetramethyl rhodamine ethyl ester in the "redistribution mode" (32).

Statistical analysis
The paired Student’s \( t \) and the 1-way ANOVA, followed by Newman–Keuls tests were used to establish the statistical significance between, respectively, different levels of apoptosis, mitochondrial membrane potential, and \([\text{Ca}^{2+}]_m \) in transfected cells and the respective scramble controls. Spearman rank and Kendall tau tests were used to establish the statistical correlation between sorcin protein levels and, respectively, histopathologic parameters and TRAP1 protein levels.

Results
The 22 kDa sorcin isoform is upregulated in human CRCs and in drug-resistant human CRC cells
The protein levels of sorcin were evaluated in a series of 59 human CRCs by immunoblot analysis, and mRNA expression of 22 kDa sorcin was assessed in a subgroup of 25 CRCs. Figure 1A shows an immunoblot analysis of sorcin in 4 CRCs, chosen as representative analytical results of all samples. Characteristics of patient are reported in Supplementary Table S1, whereas protein and mRNA levels in the whole series are reported in Supplementary Table S2. Immunoblot analysis
showed that the 22 kDa isoform of sorcin was upregulated in 28 of 59 (47.5%) carcinomas, whereas real-time reverse transcriptase PCR (RT-PCR) analysis revealed that only 5 of 14 colorectal tumors with increased 22 kDa sorcin protein levels were characterized by a concomitant upregulation of its transcript (Supplementary Table S2), suggesting that both transcriptional and posttranscriptional mechanisms are involved in the regulation of 22 kDa sorcin expression. By contrast, the 18 kDa mitochondrial isoform of sorcin was upregulated in a minority of human CRCs (11/59 tumors, 18.6%), and its upregulation was also dependent on either transcriptional or posttranscriptional mechanisms (data not shown). Interestingly, besides this different expression profile, a significant correlation was observed between the protein levels of 18 and 22 kDa sorcin isoforms (Spearman rank, \( P = 0.001 \); Kendall tau, \( P = 0.003 \)).

The protein expression of sorcin isoforms was analyzed for the major histopathologic parameters of human CRC. A significant correlation was observed between the expression of 22 kDa sorcin and either tumor grading (Kendall tau, \( P = 0.01 \)), depth of intestine wall invasion (Spearman rank, \( P = 0.04 \); Kendall tau, \( P = 0.005 \)), presence of lymph node (Spearman rank, \( P = 0.05 \); Kendall tau, \( P = 0.004 \)), or distant (Kendall tau, \( P = 0.01 \)) metastases, whereas a trend toward a positive correlation was observed between 22 kDa sorcin levels and tumor stage (Kendall tau, \( P = 0.08 \)). No statistical correlation was observed between 18 kDa sorcin levels and the same histopathologic parameters. The expression of 22 kDa sorcin was also analyzed for TRAP1 protein levels (Supplementary Table S2) because our previous studies showed that the expression of this mitochondrial TRAP1/HSP75 chaperone is increased in 65% of human CRCs (8), and a significant coexpression of the two proteins was observed in tumor specimens (Spearman rank, \( P = 0.002 \); Kendall tau, \( P = 0.00001 \)).

The major interest of our group is to identify new molecular mechanisms/targets for colon cancer chemoresistance. To this aim, sorcin expression was evaluated in HT-29 CRC cells resistant to fluorouracil (FU), oxaliplatin (l-OHP), and irinotecan (IRI), and a significant upregulation of the 22 kDa isoform was observed (Fig. 1B). Real-time RT-PCR showed a modest upregulation of 22 kDa sorcin transcript only in IRI-resistant HT-29 cells (data not shown), consistently with the relevance of posttranscriptional mechanisms in the regulation of sorcin protein expression.

The fractionation of subcellular compartments revealed that the 22 kDa sorcin band is present in the cytoplasm and in the ER (Fig. 1C and Supplementary Fig. S1), as previously reported in ventricular cardiac myocytes (15), whereas the 18 kDa isoform, known to be a TRAP1 interactor (11), is restricted to the mitochondria both in HCT-116 CRC cells (Fig. 1C) and in CRC HT-29 FU-resistant cells (Supplementary Fig. S1A), as well as in a representative case of human CRC (Supplementary Fig. S1B). Considering the functional role of sorcin in ER calcium homeostasis, the "topology" of sorcin ER localization was further evaluated through biochemical assays on the basis of protease digestion. Interestingly, the exposure to proteinase K of the ER subfraction revealed that 22 kDa sorcin is located within the ER, similarly to the molecular chaperone calnexin, a well-known ER-resident protein (ref. 33; Fig. 1D).

The 22 kDa isoform of sorcin is involved in MDR in human CRC cells

Some evidence suggests that sorcin may be responsible for inducing MDR in human tumors (17–23), but no information is
available on the specific function of the 22 kDa isoform in CRC drug resistance. Thus, pRC- or 22 kDa sorcin-HCT-116–stable transfectants (Supplementary Fig. S2A) were cultured for 48 hours in the presence of FU, IRI, or l-OHP to evaluate the rate of apoptotic cell death. Cell sensitivity to each drug is expressed as the ratio between drug-induced and vehicle-induced apoptosis in drug- and vehicle-treated cells. Ratios are calculated between rates of apoptosis induced by FU, I-OHP, and IRI was observed. Consistently, the selective downregulation of 22 kDa sorcin by siRNA (Supplementary Fig. S2B and C) enhanced the proapoptotic activity of the same three antiblastic agents by a magnitude similar to that induced by the simultaneous knockdown of both sorcin isoforms (Table 2).

Table 1. Rates of apoptotic cell death in colorectal carcinoma cells transfected with sorcin

<table>
<thead>
<tr>
<th></th>
<th>HCT-116 cells scramble</th>
<th>HCT-116 siRNA TRAP1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pRC</td>
<td>22 kDa sorcin</td>
</tr>
<tr>
<td>Vehicle</td>
<td>5.1 ± 0.2</td>
<td>6.5 ± 0.1</td>
</tr>
<tr>
<td>10 μmol/L FU</td>
<td>8.8 ± 0.5</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>20 μmol/L FU</td>
<td>21.4 ± 0.9</td>
<td>7.6 ± 0.5</td>
</tr>
<tr>
<td>10 μmol/L I-OHP</td>
<td>28.7 ± 2.4</td>
<td>9.1 ± 1.5</td>
</tr>
<tr>
<td>10 μmol/L IRI</td>
<td>27.0 ± 1.7</td>
<td>14.3 ± 1.2</td>
</tr>
<tr>
<td>Vehicle</td>
<td>4.5 ± 0.3</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td>20 μmol/L FU</td>
<td>34.8 ± 2.7</td>
<td>31.9 ± 2.9</td>
</tr>
<tr>
<td>10 μmol/L I-OHP</td>
<td>51.7 ± 3.1</td>
<td>42.3 ± 3.4</td>
</tr>
<tr>
<td>10 μmol/L IRI</td>
<td>44.4 ± 2.2</td>
<td>38.6 ± 3.7</td>
</tr>
</tbody>
</table>

NOTE: HCT-116 cells were stably transfected with pRC vector control or 22 kDa sorcin constructs and treated with FU, l-OHP, or IRI at the indicated concentrations for 48 hours or with the 3 antiblastic agents for 48 hours upon transient (siRNA) downregulation of 22 kDa sorcin or both isoforms of sorcin. Ratios are calculated between rates of apoptosis in drug- and vehicle-treated cells. P values indicate the statistical significance between the ratios of apoptosis induced by FU, I-OHP, and IRI was observed. Consistently, the selective downregulation of 22 kDa sorcin by siRNA (Supplementary Fig. S2B and C) enhanced the proapoptotic activity of the same three antiblastic agents by a magnitude similar to that induced by the simultaneous knockdown of both sorcin isoforms (Table 2).

Table 2. Rates of apoptotic cell death in colorectal carcinoma cells upon transient downregulation of sorcin

<table>
<thead>
<tr>
<th></th>
<th>Apoptosis (%±SD)</th>
<th>Ratio (±SD)</th>
<th>P</th>
<th>Apoptosis (%±SD)</th>
<th>Ratio (±SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Cyclosporine</td>
<td></td>
<td>Vehicle</td>
<td>Cyclosporine</td>
<td></td>
</tr>
<tr>
<td>Scramble</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.1 ± 0.2</td>
<td>5.4 ± 0.2</td>
<td></td>
<td>4.1 ± 0.1</td>
<td>7.4 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>FU</td>
<td>6.7 ± 0.3</td>
<td>1.6 ± 0.2</td>
<td>1.2 ± 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l-OHP</td>
<td>10.1 ± 0.2</td>
<td>2.5 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRI</td>
<td>20.3 ± 0.4</td>
<td>4.9 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>siRNA Sor22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.1 ± 0.1</td>
<td>7.4 ± 0.4</td>
<td></td>
<td>14.7 ± 0.2</td>
<td>13.0 ± 0.5</td>
<td>1.7 ± 0.2 n.s.</td>
</tr>
<tr>
<td>FU</td>
<td>10.1 ± 0.3</td>
<td>3.6 ± 0.1</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l-OHP</td>
<td>22.6 ± 0.3</td>
<td>5.5 ± 0.2</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRI</td>
<td>31.5 ± 0.4</td>
<td>7.7 ± 0.3</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>siRNA Sor18/22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.4 ± 0.2</td>
<td>7.5 ± 0.5</td>
<td></td>
<td>16.1 ± 0.3</td>
<td>13.8 ± 0.6</td>
<td>1.8 ± 0.3 n.s.</td>
</tr>
<tr>
<td>FU</td>
<td>25.2 ± 0.3</td>
<td>4.7 ± 0.2</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l-OHP</td>
<td>37.1 ± 0.5</td>
<td>6.9 ± 0.3</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRI</td>
<td>16.6 ± 0.5</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: HCT-116 cells were treated with 10 μmol/L FU, I-OHP, or IRI for 48 hours in the presence and the absence of 1 μmol/L cyclosporine A upon transient (siRNA) downregulation of 22 kDa sorcin or both isoforms of sorcin. Ratios are calculated between rates of apoptosis in drug- and vehicle-treated cells. P values indicate the statistical significance between the ratios of apoptosis induced by antiblastic agents in transfected cells and in the siRNA negative control. Abbreviation: n.s., not significant.
The downregulation of 22 kDa sorcin in drug-resistant CRC cells restores the sensitivity to antiblastic agents in vitro

We further evaluated whether the selective silencing of 22 kDa sorcin by siRNAs is able to restore sensitivity to chemotherapeutics in drug-resistant cells. To address this issue, we used wild-type HT-29 cells and HT-29 cells resistant to FU, l-OHP, and IRI, which had been previously characterized by increased expression of 22 kDa sorcin (Fig. 1B). Cells were exposed to FU, l-OHP, or IRI upon silencing of 22 kDa sorcin or both sorcin isoforms by siRNAs (Supplementary Fig. S2D). As reported in Table 3, wild-type HT-29 cells treated with FU, l-OHP, and IRI exhibited a significant increase in apoptotic rates, whereas HT-29 cells adapted to growth in the presence of the same concentrations of these agents did not exhibit any increase in apoptotic cell death. Interestingly, drug-resistant HT-29 cells, depleted of 22 kDa sorcin by siRNAs, revealed
increased rates of apoptosis upon treatment with the respective resistance antiblastic agent (Table 3).

The antiapoptotic activity of 22 kDa sorcin involves the regulation of Ca\(^{2+}\) homeostasis in the ER

Because sorcin is involved in the regulation of Ca\(^{2+}\) homeostasis in cardiomyocytes, mainly by regulating the activity of the ryanodine receptor RyRs (12, 15), we questioned whether the cytoprotective activity of 22 kDa sorcin might depend on its ability to modulate intracellular Ca\(^{2+}\) levels. To this aim, Tg, an inhibitor of the SERCA pump, was used to induce the release of Ca\(^{2+}\) from the ER, thus providing an indirect measure of Ca\(^{2+}\) levels into the ER (31). In fact, by blocking Ca\(^{2+}\) uptake from cytosol, Tg was able to induce a progressive and complete ion depletion from ER (Fig. 2A and B). Interestingly, the exposure of HCT-116 cells to FU determined changes in the ER Ca\(^{2+}\) content that were dependent on drug concentration. Indeed, after 48 hours of exposure to 10 µmol/L FU, a greater accumulation of Ca\(^{2+}\) was detected in all treatment conditions, as an initial response to this toxic stimulus (Fig. 2C). Interestingly, the Tg-induced Ca\(^{2+}\) release reached significantly higher levels in HCT-116 sorcin transfectants than in all other experimental conditions, suggesting that the upregulation of 22 kDa sorcin favors accumulation of Ca\(^{2+}\) into ER. This effect was prevented in 22 kDa sorcin-depleted cells. By contrast, the exposure of the same CRC cell lines to 40 µmol/L FU for 48 hours, a treatment that induces higher levels of apoptosis (data not shown), resulted in significant depletion of ER Ca\(^{2+}\) levels in pRC- and siRNA-transfected cells and a further increase in ER Ca\(^{2+}\) accumulation among 22 kDa sorcin transfectants (Fig. 2C). In the same experimental conditions, a significant increase in cytosolic Ca\(^{2+}\) levels was observed only in pRC- and siRNA-transfected cells exposed to 40 µmol/L FU for 48 hours (see legend to Fig. 2). Similarly, 22 kDa sorcin HCT-116 transfectants treated with 30 µmol/L l-OHP for 48 hours exhibited an increased release of Ca\(^{2+}\) from the ER compared with the respective untreated conditions (Fig. 2D). Such evidence suggests that the overexpression of 22 kDa sorcin in CRC cells induces the accumulation of Ca\(^{2+}\) into the ER and that this correlates with resistance to apoptosis.

The 22 kDa sorcin is involved in protecting from ER stress and preventing the opening of the mitochondrial transition pore

Because the perturbation of Ca\(^{2+}\) homeostasis in the ER is a major event which triggers ER stress (34), we evaluated whether a correlation between sorcin expression and ER stress could be observed in our experimental systems. A significant upregulation of 22 kDa sorcin was observed upon treatment of HCT-116 cells with 2 µmol/L Tg for 7 hours, a condition that induced ER stress (ref. 35; Fig. 3A). Accordingly, the selective downregulation of 22 kDa sorcin in HCT-116 CRC cells treated with 10 µmol/L FU resulted in reduced levels of caspase-3 and the proteolytic cleavage of the caspase-12 precursor, a molecular event which triggers apoptotic signaling in response to ER stress (ref. 35; Fig. 3B). Furthermore, the downregulation of 22 kDa or both sorcin isoforms by siRNAs resulted in increased upregulation of GRP78/BiP, a well-known molecular ER chaperone (36), in response to Tg (Fig. 3C). By contrast, the 22 kDa sorcin exhibited a protective activity toward ER stress, as revealed by the reduced levels of GRP78/BiP mRNA upon Tg stimulation compared with pRC-transfected cells (Fig. 3C). Finally, because exposure to 40 µmol/L FU resulted in a condition of ER stress with depletion of Ca\(^{2+}\) levels in the ER and a parallel increase in cytosolic Ca\(^{2+}\) (Fig. 2C), we questioned whether the localization of sorcin was modified by this treatment. Interestingly, the exposure of HCT-116 cells to 40 µmol/L FU for 48 hours resulted in a translocation of 22 kDa sorcin from the ER to the cytosolic compartment (Fig. 3D). These results suggest that the upregulation of the 22 kDa sorcin isoform prevents the ER stress response and that its cytoprotective function might depend on its role in Ca\(^{2+}\) homeostasis in the ER.

The perturbation of Ca\(^{2+}\) homeostasis in the ER and the consequent ER stress represent conditions that favor apoptosis through the opening of the mitochondrial transition pore (MTP; ref. 37); we thus questioned whether sorcin-dependent protection of apoptosis may involve the mitochondrial anti-apoptotic pathway and the modulation of mitochondrial function. To this aim, the rate of apoptosis induced by antiblastic agents was analyzed (i) in 22 kDa sorcin HCT-116 transfectants after selective downregulation of TRAP1, a mitochondrial chaperone known to antagonize the activity of cyclophilin D, an immunophilin that induces mitochondrial cell death (5), and (ii) in HCT-116 cells depleted of 22 kDa sorcin and treated with cyclosporine A, an inhibitor of MTP opening (38). Interestingly, TRAP1 interference by siRNA restored the sensitivity to FU, l-OHP, and ERI-induced cell death in 22 kDa sorcin HCT-116 transfectants (Table 1), whereas the inhibition of MTP opening by cyclosporine A prevented apoptosis induced by antiblastic agents in 22 kDa sorcin-depleted HCT-116 cells (Table 2). These results suggest that the cytoprotective activity of 22 kDa sorcin involves the modulation of mitochondrial function.

The role of 22 kDa sorcin in the modulation of mitochondrial activity was further supported by experiments aimed at measuring the mitochondrial membrane potential and calcium concentration ([Ca\(^{2+}\)]\(_{\text{m}}\)). Indeed, the silencing of 22 kDa sorcin induced a significant mitochondrial membrane hyperpolarization and a reduction in [Ca\(^{2+}\)]\(_{\text{m}}\) (Fig. 4A), whereas 22 kDa sorcin overexpression induced a significant mitochondrial depolarization and a slight mitochondrial calcium increase (Fig. 4C). These results suggest that, by modulating mitochondrial membrane potential, 22 kDa sorcin plays a crucial role in the regulation of mitochondrial calcium efflux. Interestingly, treatment with cytotoxic concentrations of l-OHP (40 µmol/L) in 22 kDa sorcin-silenced HCT-116 cells, although reducing mitochondrial calcium levels to the same extent as untreated silenced cells, restored the mitochondrial membrane potential (Fig. 4B). These effects might be responsible for greater susceptibility of 22 kDa sorcin-depleted HCT-116 cells to apoptosis induced by antiblastic agents. Consistently, the restoring of mitochondrial membrane potential in 22 kDa sorcin-silenced HCT-116 cells treated with l-OHP, together with lowering of [Ca\(^{2+}\)]\(_{\text{m}}\), might preserve metabolic cell rate and ATP production, thus contributing to apoptotic cell death.
Figure 2. ER 

Discussion

Several observations suggest that sorcin is involved in a cytoprotective pathway in human malignancies, being responsible for drug resistance (17–23). However, to our knowledge, no evidence has been reported for a role of the 22 kDa sorcin isoform in the MDR phenotype of human CRCs, whereas the functional mechanism proposed for its anti-apoptotic activity is still quite elusive. Sorcin has been extensively studied in cardiomyocytes due to its role as a Ca$^{2+}$-sensitive protein, localized in the cytosolic and membranous compartments (15) and involved in controlling...
intracellular Ca\(^{2+}\) homeostasis, regulating the activity of several Ca\(^{2+}\) transporters (12, 13, 15) and modulating excitation–contraction coupling (15, 16).

The interest of our group in studying sorcin originated from a recent observation showing that a new mitochondrial sorcin isoform (18 kDa), identified by our group, is a TRAP1 interacting protein, playing a critical role in the mitochondrial antiapoptotic pathway (11). Indeed, the Ca\(^{2+}\)-dependent interaction between TRAP1 and 18 kDa sorcin is required for sorcin mitochondrial localization/stability and seems crucial for...
TRAP1 regulation of cell survival (11). Although this observation provides mechanistic insights into the cytoprotective role of the mitochondrial sorcin isoform, suggesting that its anti-apoptotic function depends on its participation in the TRAP1 pathway (11), it does not provide any information on the cytoprotective function of the 22 kDa isoform of sorcin, which is the most abundant cellular isoform and is not a TRAP1 interacting protein (11). Here, we report that 22 kDa sorcin is upregulated in about 50% of human CRCs, and its upregulation induces protection against drug-induced apoptosis. Because a significant amount of the protein is localized within the ER and previous studies have shown that sorcin is involved in modulating the ryanodine receptor RyRs (12, 15), an ER-resident protein responsible for Ca\(^{2+}\) efflux from the ER (39), we questioned whether the cytoprotective activity may be mediated by its ability to regulate Ca\(^{2+}\) homeostasis in the ER. This hypothesis is in agreement with several lines of evidence arguing in favor of a role of ER Ca\(^{2+}\) content in the resistance to stress and apoptotic stimuli (34, 40). Our results suggest that the treatment of sorcin overexpressing HCT-116 CRC cells with FU and I-OHP induced a dramatic response to Tg, an inhibitor of SERCA, responsible for the depletion of ER Ca\(^{2+}\) content and, thus, a widely used tool for measuring Ca\(^{2+}\) levels in the ER (31). Therefore, it is likely that the upregulation of 22 kDa sorcin favors the accumulation of Ca\(^{2+}\) into the ER, possibly by inhibiting RyRs receptors (15), and this may be responsible for the cytoprotective function exerted by the calcium-binding protein. Indeed, sorcin-dependent accumulation of Ca\(^{2+}\) into the ER seems to be critical in preventing ER stress and in protecting tumor cells from apoptosis, because 22 kDa sorcin is upregulated in response to conditions of ER stress. Furthermore, ER-associated sorcin translocates to cytosol under conditions of high apoptotic cell death, thus determining greater ER Ca\(^{2+}\) depletion. By contrast, the selective downregulation of 22 kDa sorcin results in the activation of caspase-3 and caspase-12, in the upregulation of GRP78/FBP, and in the sensitization of tumor cells to drug-induced apoptosis by lowering the threshold of MTP opening. Indeed, downregulation of 22 kDa sorcin is associated with hyperpolarization of the mitochondrial membrane and reduced calcium content in mitochondria which, in turn, might promote drug-induced apoptosis.

Such evidence is consistent with previous studies suggesting that modulation of Ca\(^{2+}\) homeostasis is a crucial step in the regulation of cell response to stress conditions and in favoring drug resistance in tumors (40, 41). Indeed, neuronal cells exposed to oxygen and glucose deprivation activate the Na\(^+\)/Ca\(^{2+}\) exchanger NCX1, which is known to be a target protein of sorcin (14), and this is correlated with accumulation of Ca\(^{2+}\) into the ER, and prevention of ER stress and apoptosis (42). Furthermore, increased levels of Ca\(^{2+}\) in the ER have also been correlated with MDR phenotypes in cancer cells (41, 43). Thus, it is intriguing to speculate that sorcin isoforms are both involved in regulating Ca\(^{2+}\) homeostasis in separate cell compartments and that this function is relevant for their anti-apoptotic activities in tumor cells. Indeed, the 2 proteins, though translated from 2 independent transcripts, are almost identical in their structure because they share 5 EF-hand motifs, typical calcium-binding sites, and diverge at the N-terminus due to the absence of 15 amino acids in the mitochondrial isoform. Although the role of 18 kDa sorcin in the regulation of mitochondrial calcium homeostasis needs to be confirmed by further studies, it is likely that the 22 kDa sorcin isoform is one of several ER stress proteins involved in the control of Ca\(^{2+}\) levels in the ER, preventing ER stress and the subsequent apoptotic events, and may co-operate with 18 kDa sorcin in controlling Ca\(^{2+}\) homeostasis in mitochondria contributing to the regulation of the MTP opening. This hypothesis, although still preliminary, sheds some light on the relevance of the TRAP1/18 kDa sorcin interaction and the role of this sorcin isoform in the TRAP1 cytoprotective pathway. Indeed, the activity of TRAP1 chaperone may be crucial for 18 kDa sorcin transport/stability in mitochondria because sorcin lacks a mitochondrial localization sequence (11), and this interaction contributes to sorcin-induced regulation of Ca\(^{2+}\) homeostasis in mitochondria and Ca\(^{2+}\)-dependent MTP regulation. In addition, recent evidence suggests that TRAP1 may be also involved in the regulation of the unfolded protein response induced by ER stress (44, 45), suggesting a potential cross-talk between mitochondria and the ER stress response pathways (46). This hypothesis is consistent with the observation that, in our series of human CRCs, TRAP1 expression correlates with 22 kDa sorcin levels. Thus, it is likely that 22 kDa sorcin and TRAP1 are components of a coordinated adaptive response of tumor cells to counteract ER stress conditions and apoptotic signaling.

To our knowledge, this study is the first evidence of a role played by sorcin in resistance to FU, IRL, and I-OHP, 3 chemotherapeutics that represent the backbone of human CRC treatment (47). Apoptosis assays upon selective RNAi knockdown of 22 kDa sorcin in drug-resistant CRC cells suggest that targeting sorcin may form the basis for a novel therapeutic strategy for improving the efficacy of chemotherapy in CRCs. Indeed, previous studies in several human tumor cell models evaluated verapamil, an agent blocking Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels, and suggested that such a strategy may antagonize P-glycoprotein–mediated MDR in vitro, but it provided uncertain results in clinical trials (41). However, because sorcin overexpression has been correlated with upregulation of MDR1/P-glycoprotein (22, 23) and because P-glycoprotein–dependent MDR phenotype seems to be related to intracellular Ca\(^{2+}\) homeostasis (41), our results suggest that targeting 22 kDa sorcin may represent an innovative strategy to prevent Ca\(^{2+}\) accumulation in the ER and likely revert the MDR phenotype. Thus, further studies are needed to confirm the relationship between sorcin, Ca\(^{2+}\) homeostasis, and the MDR phenotype and to design agents able to modulate ER Ca\(^{2+}\) through the inhibition of 22 kDa sorcin activity. Interestingly, several signaling pathways induced by ER stress are currently regarded as novel molecular targets for cancer therapy (34).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
Grant Support
This work was supported by AIRC (IG07890 to M. Landriscina and F. Esposito), MIUR (PRIN 2008 to M. Landriscina and F. Esposito), and Fondazione Berlucchi (M. Landriscina and F. Esposito).

References

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received June 29, 2011; revised September 15, 2011; accepted October 6, 2011; published OnlineFirst November 3, 2011.
47. O’Neill BH, Goldberg RM. Chemotherapy for advanced colorectal cancer: let’s not forget how we got here (until we really can). Semin Oncol 2005;32:35–42.
Sorcin Induces a Drug-Resistant Phenotype in Human Colorectal Cancer by Modulating Ca\(^{2+}\) Homeostasis

Francesca Maddalena, Gabriella Laudiero, Annamaria Piscazzi, et al.


**Updated version**
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-11-2172

**Supplementary Material**
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2011/11/03/0008-5472.CAN-11-2172.DC1

**Cited articles**
This article cites 46 articles, 10 of which you can access for free at:
http://cancerres.aacrjournals.org/content/71/24/7659.full#ref-list-1

**Citing articles**
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/71/24/7659.full#related-urls

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/71/24/7659.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.