SRT1720 Induces Lysosomal-Dependent Cell Death of Breast Cancer Cells

Tyler J. Lahusen and Chu-Xia Deng

Abstract

SRT1720 is an activator of SIRT1, a NAD⁺-dependent protein and histone deacetylase that plays an important role in numerous biologic processes. Several studies have illustrated that SRT1720 treatment could improve metabolic conditions in mouse models and in a study in cancer SRT1720 caused increased apoptosis of myeloma cells. However, the effect of SRT1720 on cancer may be complex, as some recent studies have demonstrated that SRT1720 may not directly activate SIRT1 and another study showed that SRT1720 treatment could promote lung metastasis. To further investigate the role of SRT1720 in breast cancer, we treated SIRT1 knockdown and control breast cancer cell lines with SRT1720 both in vitro and in vivo. We showed that SRT1720 more effectively decreased the viability of basal-type MDA-MB-231 and BT20 cells as compared with luminal-type MCF-7 breast cancer cells or nontumorigenic MCF-10A cells. We demonstrated that SRT1720 induced lysosomal membrane permeabilization and necrosis, which could be blocked by lysosomal inhibitors. In contrast, SRT1720-induced cell death occurred in vitro irrespective of SIRT1 status, whereas in nude mice, SRT1720 exhibited a more profound effect in inhibiting the growth of allograft tumors of SIRT1 proficient cells as compared with tumors of SIRT1-deficient cells. Thus, SRT1720 causes lysosomal-dependent necrosis and may be used as a therapeutic agent for breast cancer treatment. Mol Cancer Ther; 14(1): 183–92. ©2014 AACR.

Introduction

Breast cancer is the most common type of cancer to affect American women. It is estimated that over 200,000 women will develop breast cancer in 2014 and about 40,000 will die from the disease (1). There are many treatment options for women with ER+/PR+/HER2⁺ luminal-type breast cancers, but for basal-type ER⁻/PR⁻/HER2⁻ breast cancers (triple-negative breast cancers, TNBC), there are few options currently available (2, 3). Therefore, novel therapeutics targeting TNBCs and other breast cancers that are resistant to current therapies are desperately needed. In this study, we wanted to elucidate whether the small-molecule compound SRT1720 is a potential therapeutic agent for breast cancer. SRT1720 was initially identified as an activator of SIRT1, a NAD⁺-dependent deacetylase, which was shown to positively affect glucose and lipid homeostasis (4–7). However, more recently other researchers have demonstrated that SRT1720 has SIRT1-independent effects and does not directly activate SIRT1 (8).

Anticancer drugs may cause cell death either through the process of apoptosis or necrosis (9, 10). Apoptosis, also known as programmed cell death, results in the activation of caspases to cause membrane swelling and DNA fragmentation (11). Necrosis is another type of cell death that is not dependent on caspase activation but results from an alternative mechanism such as lysosomal membrane permeabilization (LMP), thus causing cell proteolysis and membrane disruption (12). In a previous study, it was shown that SRT1720 induced apoptosis of myeloma cells (13), while another study reported that SRT1720 could promote tumor cell migration and lung metastasis in mice (14). Neither study investigated whether such effects of SRT1720 were dependent on SIRT1. Therefore, it is inconclusive whether the effect of SRT1720 on cancer cells is dependent on SIRT1.

To determine the effect of SRT1720 in breast cancer and study its underlying mechanism, we treated SIRT1-deficient and proficient breast cancer cells with SRT1720. Our data demonstrated that SRT1720 caused lysosomal-dependent cell death in breast cancer cells in vitro irrespective of their SIRT1 status. SRT1720 could also inhibit the growth of allograft tumors in nude mice that was partially mediated by SIRT1. These data reveal that SRT1720 has both SIRT1-dependent and -independent functions and may potentially be a therapeutic agent for the treatment of breast cancer cells.

Materials and Methods

Cell lines and reagents

All human breast cancer cell lines (MCF-7, T47D, SKBR3, MDA-MB-231, SUM149, HS578T, and BT-20) and the A549 lung adenocarcinoma cells were obtained from ATCC and cultured with DMEM supplemented with 10% FBS (Sigma) and 1% L-glutamine (Invitrogen). All cell lines from ATCC are authenticated. Mouse mammary tumor cells were from MMTV-neu mice (Neu) and from Braf<sup>V600E</sup> p53<sup>−/−</sup>;MMTV-Cre mice (69), respectively (15, 16). MCF10A immortalized mammary epithelial cells were obtained from ATCC and cultured with DMEM/F12 (1:1; Invitrogen) supplemented with 5% horse serum.

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Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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concentration of the supernatant was quantified by luciferase activity. 

For production of lentiviral SIRT1 shRNA, 293T cells (3 × 10⁶) were seeded in 100-mm dishes and 1 mL of viral supernatant was added to 7 mL of medium after cell attachment. The cells were incubated with the Bio-Rad Genepherm at 37°C for 15 minutes.

Preparation and transduction of lentiviral-delivered shRNA

For transduction of lentiviral shRNA, pLKO.1 lentiviral vectors targeting SIRT1 were obtained from Sigma. The lentiviral SIRT1 shRNA clone, pLKO.1-Scrambled, was obtained through the plasmid repository Addgene (18). For production of lentiviral particles expressing SIRT1 shRNA, 293T cells (3 × 10⁶) were seeded in 100 mm dishes. After the cells attached, the transfection complex was prepared as follows according to the manufacturer’s instructions for X-tremeGENE9 (Roche Applied Science). Of note, 3 μg of the pLKO.1-SIRT1 shRNA vector was added to 18 μL of X-tremeGENE9 in 500 μL DMEM along with 3 μg pCMV-dR8.2 dvpr packaging vector and 0.375 μg pCMV-VSV-G envelope vector. The packaging and envelope vectors were created by the laboratory of Robert Weinberg (19) and obtained through Addgene. The transfection complex was added to the cells for 24 hours of incubation, the cells were washed with medium, and 10 mL of fresh medium was added for another 24 hours. The medium containing lentiviral particles was then collected, centrifuged at 14,000 rpm at 4°C for 15 minutes, filtered through a 0.45 μm polyethersulfone syringe filter (EMD Millipore), and aliquots were stored at −80°C. For transduction of lentiviral particles, MDA-MB-231 (5 × 10⁵) cells were seeded in 100-mm dishes and 1 mL of viral supernatant was added to 7 mL of medium after cell attachment. The cells were transduced for 24 hours in the presence of polybrene (8 μg/mL; Sigma). Cells stably expressing SIRT1 shRNA were selected for 48 hours in the presence of puromycin (2 μg/mL; Sigma) before plating for experiments.

Western blot analysis

Cells were harvested from subconfluent plates and whole-cell lysates were prepared for immunoblot analysis. Cells were washed with cold PBS and lysed with lysis buffer containing: 1% NP-40, 50 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl, 10% glycerol, 50 mmol/L NaF, 2 mmol/L EGTA, 2 mmol/L EDTA, 1 μg/mL Pepstatin A, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 100 μg/mL AEBSF, and 1 mmol/L sodium orthovanadate. The lysate was centrifuged at 14,000 rpm at 4°C for 10 minutes and the protein concentration of the supernatant was quantified with the Bio-Rad protein assay reagent (Bio-Rad Laboratories). Protein lysates (25–50 μg) were prepared in SDS-loading buffer containing the reducing agent β-mercaptoethanol (Sigma), heated at 95°C for 4 minutes, resolved by SDS-PAGE on 4% to 12% Tris-glycine gels (Invitrogen), and then transferred to polyvinylidene difluoride Immobilon-P membranes (EMD Millipore). The membranes were blocked with 4% nonfat milk in PBS for 30 minutes, incubated for 2 hours with primary antibodies diluted in 1% milk in PBS, washed three times with PBS-T (PBS, 0.1% Tween-20), and then incubated with either an anti-rabbit or anti-mouse horseradish peroxidase-conjugated secondary antibody (GE Healthcare) for 45 minutes. The blots were washed three times with PBS-T and the protein bands were detected on enhanced chemiluminescence film (Denville Scientific) with the Immobilon chemiluminescence detection reagent (EMD Millipore). Proteins were detected with the following antibodies: monoclonal anti-SIRT1 (1F3), LC3 (Cell Signaling Technology), and monoclonal anti-β-actin (Sigma).

Cell viability and apoptosis assays

For cell viability assays, MDA-MB-231 cells were plated at 15,000 cells/well in 24-well plates. The day after plating, SRT1720 was added to the cells for the designated time and concentration. Cell viability was assessed by methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay. A 0.5-mg/mL solution of Thiazolyl Blue (Sigma) in phenol-free DMEM was added to cells at 37°C for 1 hour. The substrate was then dissolved in isopropanol and absorbance was measured with a spectrophotometer at 570 nm. Apoptosis/necrosis was measured with the annexin V/propidium iodide staining kit from Sigma. For necrosis measurements alone, cells were trypanosized, washed in PBS, and incubated with 1 μg/mL of propidium iodide for 10 minutes. Both annexin V and propidium iodide staining were detected by FACs analysis with a FACScalibur flow cytometer (BD Biosciences).

Acridine orange and Lysotracker Red staining

Cells were incubated at 37°C for 15 minutes with 1 μg/mL of acridine orange (Sigma). The cells were washed in PBS and observed with a FACScalibur flow cytometer in both the red (FL-2) and green (FL-1) channel. For Lysotracker Red staining, cells were incubated with 100 nmol/L Lysotracker Red (Invitrogen) at 37°C for 15 minutes.

In vivo tumor study

Nude cells (1 × 10⁶) were injected into two sites of the mammary fat pads of 5 nude mice each for vehicle and SRT1720 treatment. There were a total of 10 tumor injection sites per treatment. SRT1720 (40 mg/kg/d) was delivered by intraperitoneal injection every day for the duration of the treatment. SRT1720 was prepared in 40% PEG300 and 0.5% Tween 80 in water.

Statistical analysis

Data were analyzed using Prism GraphPad (GraphPad software, Inc.). Results are presented as the mean ± SEM from independent triplicate samples. A P value of less than 0.05 was considered to be statistically significant and designated as P < 0.05 (*). P < 0.01 (**), or P < 0.001 (***). For multiple comparisons, ANOVA followed by Bonferroni posttests was used. Otherwise, the t test was used for comparison of two treatments.

Results

SRT1720 inhibits the growth of breast cancer cells in vitro in a SIRT1-independent manner

SRT1720 was previously shown to inhibit the proliferation of myeloma cell lines through the induction of apoptosis (13). In our study, we determined whether SRT1720 affected the viability of breast cancer cell lines. Human and mouse breast cancer cells,
MCF10A immortalized human mammary epithelial cells, and A549 lung and HCT116 colon adenocarcinoma cell line were treated with various concentrations of SRT120 for 24 hours and assessed for cell viability by MTT assay. The viability of MDA-MB-231 and BT20 basal-type breast cancer cells decreased by more than 80% with 5 μmol/L of SRT1720, whereas the viability of MCF-7 luminal-type cells only decreased by 20% with 20 μmol/L of treatment (Fig. 1A). The viability of MCF10A immortalized mammary epithelial cells was not affected up to 20 μmol/L of SRT1720 treatment. Therefore, MDA-MB-231 cells were used for further experiments as they were more sensitive to SRT1720 treatment. Next, we treated MDA-MB-231 cells with a dose range of SRT1720 from 2.5 to 10 μmol/L to determine the concentration, which begins to affect cell viability. The viability of MDA-MB-231 cells was decreased by 66% to 86% with a dose range from 5 to 10 μmol/L (Fig. 1B). Therefore, MDA-MB-231 cells treated with SRT1720 have a very narrow dose response. The sensitivity of MDA-MB-231 cells to 5 μmol/L of SRT1720 treatment was also affected by the plating density of the cells. If the cell density was doubled from 15,000 (low) to 30,000 (high) cells per well in a 24-well plate, there was no effect on cell viability; however, 10 μmol/L of SRT1720 decreased the viability of cells plated at both low and high density (Supplementary Fig. S1A). To determine whether a secreted factor could affect the sensitivity of MDA-MB-231 cells to SRT1720, we collected cell media from cells plated at a high density and added this medium with or without SRT1720. Interestingly, MDA-MB-231 cells cultured in this media were resistant to SRT1720 treatment (Supplementary Fig. S1B). Therefore, a secreted factor from MDA-MB-231 cells grown at a high density could affect the sensitivity of cells to SRT1720 treatment although the identity of this secreted factor is currently unknown.

Previous reports have shown that SRT1720 exerts biologic effects through the sirtuin deacetylase SIRT1 (4, 20). We determined whether the effect on cell viability by SRT1720 treatment was dependent on SIRT1 expression. We measured cell viability after SIRT1 knockdown with lentiviral-transduced SIRT1 shRNA in MDA-MB-231 cells or SIRT1 knockout MEFs. SRT1720 treatment of both MDA-MB-231 sh-control and sh-SIRT1 knockdown cells decreased cell viability by more than 90%, which was also observed in immortalized WT and Sirt1−/− MEFs (Fig. 1C and D). Therefore, SRT1720 decreases the viability of MDA-MB-231 cells and MEF cells independently of SIRT1 expression. We also compared the effect of resveratrol, another reported activator of SIRT1 (21, 22), on the viability of MDA-MB-231 cells. There was a 30% decrease in the viability of sh-control MDA-MB-231 cells treated with 80 μmol/L of resveratrol as compared with a decrease of 47% for sh-SIRT1 knockdown cells, thus suggesting that SIRT1 is not required for resveratrol-induced cell death (Supplementary Fig. S2). We next determined whether the effect of SRT1720 on breast cancer cells was dependent on other members of the sirtuin family by cotreatment with the pan-sirtuin inhibitor nicotinamide. Our results showed that other sirtuin members also do not have a role in SRT1720-induced cell death (Supplementary Fig. S3).

SRT1720 decreases the viability of breast cancer cells by increased necrosis

A decrease in cell viability may result from decreased cell-cycle progression or through increased cell death. The cause of a reduction in cell viability was determined by measuring apoptosis and necrosis. Cells were treated with SRT1720 for 8 hours and apoptosis/necrosis was measured by annexin V/propidium iodide staining, respectively. After 5 μmol/L of SRT1720 treatment, 1% of the cells were positive for early apoptosis/necrosis (only annexin V positive) and 12% of the cells were positive for late apoptosis/necrosis (double positive for annexin V and propidium iodide; Fig. 2A). Measurement of necrosis with propidium iodide alone showed a 13% and 24% increase in necrotic cells with 5 and 10 μmol/L SRT1720, respectively, after 8 hours of SRT1720 treatment (Fig. 2B). The pan-caspase inhibitor ZVAD was used to determine whether apoptosis had any role in SRT1720-induced cell death. There was a 74% reduction in cell viability in SRT1720-treated cells; however, cotreatment...
of MDA-MB-231 cells with ZVAD did not reduce cell death caused by SRT1720 treatment (Fig. 2C). Also, SRT1720 did not cause caspase-3 cleavage as was observed with the proteosome inhibitor bortezomib (Fig. 2D).

SRT1720 caused the formation of acidic vacuoles and an increase in autophagy markers

Treatment of MDA-MB-231 breast cancer cells with SRT1720 for 8 hours resulted in the formation of acidic vacuoles (Fig. 3A) as detected by acridine orange staining. Acridine orange will emit red fluorescence in a low pH environment, which can be used to measure acidic vacuoles. Autophagy is a cellular process by which the cell catabolizes its own cytoplasmic organelles during nutritional deprivation. It is mainly a survival pathway, which may result in cell death during prolonged nutrient deprivation. One of the markers for autophagy is the autophagosome membrane marker LC3 that is converted from LC3-I to LC3-II through lipidation involving both ATG7 and ATG3, which leads to LC3 fusion with autophagosome membranes (23). We generated MDA-MB-231 cells with stable expression of LC3-GFP to measure the effect of SRT1720 on autophagy by observation of GFP puncta formation. Treatment with SRT1720 resulted in an increase in the number of cells with an average of eight GFP puncta per cell (Fig. 3B). An immunoblot analysis of LC3 will show an increase in the lower LC3-II band during autophagy. We observed that treatment with SRT1720 caused an increase in the autophagy marker LC3-II, and this was decreased in sh-SIRT1 knockdown MDA-MB-231 cells (Fig. 3C). This validates a previous study demonstrating that SIRT1 is involved in the regulation of autophagy (24). Next, we determined whether blocking the PI3K pathway responsible for initiation of autophagy would affect autophagy induction by SRT1720. Cotreatment of MDA-MB-231 cells with the type-III PI3K inhibitor, 3-MA, resulted in decreased LC3-II formation caused by SRT1720 treatment (Fig. 3D). Also, LC3-II formation was reduced in Sirt1 and Atg7 knockout MEFs (Fig. 3E). Therefore, SRT1720 increases the formation of the autophagy marker, LC3-II, through SIRT1 and ATG7.

Then we determined whether blocking autophagy induction by SRT1720 with 3-MA or Atg7 knockout cells could affect cell death. MDA-MB-231 cells treated with either SRT1720 or 2.5 mmol/L 3-MA resulted in a 65% and 18% decrease in cell viability, respectively. However, cotreatment of 3-MA with SRT1720 did not reduce SRT1720-induced cell death (Fig. 3F). In addition, treatment of WT MEFs with 5 mmol/L of SRT1720 resulted in a 22% decrease in cell viability, whereas the number of Atg7 knockout cells decreased by 80% (Fig. 3G). Therefore, these results suggest that autophagy is increased during SRT1720 treatment due to cellular stress as a mechanism to promote cell survival.
SRT1720 induces lysosomal membrane permeabilization

Necrotic cell death may be caused by permeabilization of the lysosomal membrane (12). LMP causes the release of cathepsins and other hydrolases from the lysosomal lumen to the cytosol (25). To determine whether the death of MDA-MB-231 cells was caused by permeabilization of the lysosomal membrane, cells were incubated with either acridine orange or LysoTracker Red. Acridine orange and LysoTracker Red normally concentrate in the lysosomal compartment and emit red fluorescence, but upon permeabilization, these dyes will diffuse out of the lysosome (12). In the case of acridine orange, the lysosomal vacuoles become yellow as a result of an increase in the pH. After treatment of MDA-MB-231 cells with SRT1720 for 16 hours, the cells were incubated with acridine orange and observed by microscopy. SRT1720 caused an increase in yellow stained cells, therefore indicating an increase in lysosomal membrane permeability (Fig. 4A). An analysis of the cells by FACS showed that there is a dose-dependent decrease in the percentage of red fluorescent cells with SRT1720 treatment. The percentage of red cells decreased by 68% with 7.5 μmol/L and 95% with 10 μmol/L of SRT1720 (Fig. 4B). We also incubated the cells with LysoTracker Red and observed a 66% and 90% decrease in LysoTracker Red staining by FACS analysis after treatment with 7.5 and 10 μmol/L of SRT1720, respectively (Fig. 4C).

Lysosomal inhibitors block SRT1720-induced cell death

As a result of our observation that SRT1720 increases LMP, we next determined whether inhibitors of lysosomal...
Acidiﬁcation could affect SRT1720-induced cell death. The lysosomotropic alkalinization agents, ammonium chloride, bafilomycin A1, and chloroquine, affect lysosomal acidiﬁcation through different mechanisms. Ammonium chloride directly neutralizes lysosomal pH, bafilomycin A1 is an inhibitor of the V-ATPase that pumps protons into the lysosome, and chloroquine blocks the fusion of the lysosome and autophagosome (23, 26, 27). We assessed the viability of MDA-MB-231 cells treated with SRT1720 and lysosomal inhibitors. We observed that ammonium chloride, bafilomycin A1, and chloroquine could signiﬁcantly reduce SRT1720-induced cell death. Treatment with SRT1720 alone resulted in a 79% decrease in viable cells, whereas cotreatment with ammonium chloride, bafilomycin A1, and chloroquine resulted in only a 23%, 20%, and 30% decrease in cell viability, respectively (Fig. 5A). We also observed that ammonium chloride could significantly reduce the death of Hs578T breast cancer cells caused by SRT1720 treatment (Supplementary Fig. S4). There was an 84% decrease in cell viability with 10 μmol/L of SRT1720 treatment (Fig. 5A). A decrease in red fluorescent cells with 7.5 (68%) and 10 (95%) μmol/L of SRT1720 treatment (***, P < 0.001). There was a decrease in red fluorescent cells with 7.5 (68%) and 10 (95%) μmol/L of SRT1720 treatment (***, P < 0.001).

Ammonium chloride could also inhibit SRT1720-induced cell necrosis (Fig. 5B) and LMP of MDA-MB-231 cells (Fig. 5C). There was a 41% increase in necrotic cells with SRT1720 and only 3% in combination with ammonium chloride. LysoTracker Red–positive cells decreased 76% with SRT1720 treatment and only 1% in combination with ammonium chloride. We then determined whether ammonium chloride treatment could affect SRT1720-induced changes in autophagy and protein homeostasis. MDA-MB-231 cells were treated with SRT1720 and/or ammonium chloride, and markers for autophagy and cellular protein stress were assessed. LC3-II induction by SRT1720 could not be blocked by ammonium chloride (Fig. 5D). An increase in LC3-II may be caused by increased autophagy or inhibition of autophagy. The protein p62/SQSTM1 is a ubiquitin binding protein that binds to LC3-II and during increased autophagy is normally degraded after fusion of the autophagosome and lysosome (23). Therefore, p62 has been used as a marker for autophagic ﬂux. Treatment of MDA-MB-231 cells with SRT1720 decreases the level of p62 protein, which indicates an increase in the rate of autophagy by SRT1720, whereas ammonium chloride inhibits SRT1720-induced p62 degradation by inhibiting lysosome function (Fig. 5D). We also determined whether SRT1720 caused activation of the endoplasmic reticulum (ER) stress pathway. ER stress is caused by a malfunction in protein processing in the ER lumen, which leads to an increase in unfolded/misfolded proteins (28). This will cause a block in protein translation by stress-kinase–induced phosphorylation of the eukaryotic initiation factor 2 alpha (eIF2α) and an induction of genes necessary for the proper folding of proteins including activating transcription factor 3 (ATF3; ref. 29). As a result of SRT1720 treatment, the phosphorylation of eIF2α and protein level of ATF3 was increased (Fig. 5D). Also, the cells were cotreated with ammonium chloride to determine whether the inhibition of SRT1720-induced death could be through the...
ER stress pathway. There was a slight reduction in eIF2α phosphorylation with ammonium chloride cotreatment; however, the induction of ATF3 with SRT1720 was completely inhibited. This observation suggests that the induction of eIF2α phosphorylation and enhanced ATF3 expression level by SRT1720 treatment may be through a different mechanism. We then determined whether SRT1720-induced cell death was dependent on ATF3. The viability of WT and Atf3−/− MEFs was measured after treatment with SRT1720; however, both cells were equally affected by SRT1720 treatment (Supplementary Fig. S5).

SRT1720 inhibits breast cancer cell growth in vivo

We have shown that SRT1720 inhibits the growth of both human and mouse breast cancer cells in vitro, so we next tested the effect of SRT1720 on tumor growth in mice. We were limited in the availability of SRT1720 so we used neu cells, a tumor cell line derived from a mammary tumor of MMTV-neu transgenic mouse (15), which grow faster as tumor xenografts in human and mouse breast cancer cells

Discussion

SIRT1 is the initial member of the 7-member sirtuin family of protein and histone deacetylases (7, 31, 32). Numerous studies have revealed important functions of SIRT1 in many biologic processes including cell proliferation, differentiation, apoptosis, senescence, metabolism, calorie restriction, lifespan regulation, and tumorigenesis (5, 6, 33–39). Although SIRT1 was initially considered as a tumor promoter in some studies, recent work has uncovered a tumor suppressor function of SIRT1, especially from
analyzing SIRT1-mutant mouse models (17, 30, 40). Although SIRT1 might have both tumor promoter and tumor suppressor activity depending on the tissue context (41–43), a few studies have tested the effectiveness of a SIRT1 activator, SRT1720, for cancer therapy. These studies demonstrated that SRT1720 could serve as an inhibitor of myeloma (13); however, it could also promote tumor cell migration and lung metastasis in mice (14).

In addition, there have been conflicting data on the use of SRT1720 as a direct activator of SIRT1 (8).

In the present study, we sought to determine whether SRT1720 could affect breast cancer cells in vitro and in vivo in a SIRT1-dependent manner. We observed that SRT1720 caused increased cell death in multiple breast cancer cell lines and other tumor types. In the panel of breast cancer cell lines that we tested, the basal-type cells including MDA-MB-231 were more sensitive to SRT1720 than luminal-type MCF-7 or non-transformed cells. However, unexpectedly the increased cell death with SRT1720 treatment in vitro was independent of SIRT1 levels. We also treated mice with SRT1720 in which neu mouse mammary tumor cells were grown in the mammary fat pad. SRT1720 also inhibited the growth of neu tumors, but in contrast with in vitro, this effect was partially dependent on SIRT1. It is unclear why there was a difference but it may be due to the function of SIRT1 in the stromal environment. It will be important to explore the role of SIRT1 and the tumor extracellular environment. From our in vitro experiments, we determined that SRT1720 caused cell death by necrosis but not apoptosis. We observed that SRT1720 activated several cellular stress pathways including autophagy and the unfolded protein response. However, the effect of SRT1720 was independent of the activation of these pathways because inhibition of either autophagy or the unfolded protein response through either chemical or genetic means did not inhibit SRT1720-induced cell death. Interestingly, we found that MDA-MB-231 cells are more resistant to SRT1720 treatment when cultured with conditioned media from cells plated at a high density. This observation suggests that a secreted factor from MDA-MB-231 cells may attenuate the effect of SRT1720. Future studies should be directed to identify this secreted factor as it may help to further understand the mechanism of action of SRT1720.

We identified LMP as the cause of SRT170-induced cell death. This could be verified with three different chemical modifiers of lysosomal function including bafilomycin A1, chloroquine, and ammonium chloride, which could all inhibit LMP and cell death caused by SRT1720. It has been established that cancer cells exhibit structural and functional changes in their lysosomes as compared with normal cells that allow cancer cells to survive (44). Therefore, targeting lysosomes to induce cell death has emerged as a possible target for cancer therapy to overcome apoptosis-resistant cells and drug resistance to chemotherapy (31). Many therapeutic agents have been reported to effect the death of cancer cells through modulation of lysosome function (25). Therefore, SRT1720 may serve as a useful agent for studying lysosomal-dependent cell death in cancers, which may eventually lead to the development of effective therapeutic approaches for cancer prevention and treatment.
Disclosure of Potential Conflicts of Interest  
No potential conflicts of interest were disclosed.

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Conception and design: T.J. Lahusen, C.-X. Deng  
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