Original Article

Allied Medical Research Journal
Vol: 4 No.1: 13-21

ML-SA5-Induced Cytotoxicity and Alterations in Lysosomal and Mitochondrial Activity in Triple-Negative Breast Cancer Cells

Alfred Henry [1], Rehan Imad[1], Saira Amir[2], Shafaq Saeed Roghay[3], Amna Bibi[1], Salman Ahmed Khan[6]

[1]*Ziauddin University, Karachi, Pakistan*
[2]*COMSATS University Islamabad, Pakistan*
[3]*Baqai Medical University, Karachi, Pakistan*
[4]*Dow University of Health Sciences (DUHS), Karachi, Pakistan*

ABSTRACT

Background: Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype that expresses neither estrogen receptors, progesterone receptors, nor HER2; therefore, limiting the current available treatments. Recent evidence shows that the lysosomal cation channel TRPML1 is overexpressed in TNBC cells whereas minimal in normal cells hence can be targeted for anticancer therapy. The objective of the present study is to determine whether Mucolipin synthetic agonist 5 (ML-SA5), a small-molecule TRPML1 agonist, selectively induces cell death in MDA-MB-231 cells and to elucidate the cellular pathways involved in this process

Methods: This study was conducted in MDRL 1 and 2 Lab, Ziauddin University, Clifton campus and this is in-vitro experimental study completed in 8 months. The cytotoxicity of MDA-MB 231 cells exposed to various doses of ML-SA5 was studied over a different time duration using the MTT assay. Cell death was further analyzed by propidium iodide and annexin V-FITC staining. Changes in mitochondrial and lysosomal activity and intracellular reactive oxygen species were evaluated.

Results: ML-SA5 induced dose-dependent cytotoxicity with IC_{50} values in the lower micromolar range (6.8 μM). PI staining indicated also confirmed cell death, with minimal apoptosis. Mitochondrial staining revealed altered morphology and compromised function. Lysosomal labeling showed enlarged lysosomes suggesting impaired lysosomal integrity. A significant rise in ROS levels was observed, indicating oxidative stress.

Conclusion: The activation of TRPML1 by ML-SA5 leads to increased oxidative stress and damage to mitochondria and lysosomes, resulting in cell death in TNBC cells.

Keywords: *Breast neoplasms, Lysosomes, Molecular targeted therapy, Mitochondria, Reactive oxygen species, Triple-negative necrosis*

Received: August 6, 2025**; Revised:** November 12, 2025**; Accepted:** December 23, 2025

Corresponding Email: rehan.imad@zu.edu.pk

DOI: https://doi.org/10.59564/amrj/04.01/002

INTRODUCTION

Breast cancer is one of the leading causes of morbidity and mortality among women worldwide, representing a significant and major health challenge both nationally and internationally. The rising incidence and long-term management of breast cancer place substantial pressure on healthcare systems globally, extending beyond specialized cancer treatment facilities[1]. Most tumors are originated in the epithelial cells lining the ducts or lobules of the breast, where malignant changes gradually developed and ultimately led to invasive disease. Invasive ductal carcinoma is by far the dominant subtype, responsible for about 80 percent of diagnoses[2]. Breast cancer has now become more common than lung cancer among population with 11.7 new cases per 100,000 population and a mortality rate of 6.9 percent making it the fifth overall cause of cancer associated mortality in the world[3]. The disease does not have a homogenous structure; it is manifested in a variety of molecular forms.

Among all cases of breast cancer, triple-negative breast cancer (TNBC) is the difficult to treat and it comprises 10 to 20 percent of the total cases[4]. TNBC tends to grow quickly, responds poorly to standard treatments, and has the highest chance of relapse. About 46 percent of TNBC patients may develop metastases, and most of the deaths occur within five years because no targeted drugs are widely available. Once the cancer has spread, the average remaining time for patients shrinks to only 13.3 months[5].

Lysosomes are membrane bound organelles containing digestive enzymes. They are involved in destruction of macromolecules generating monomers, degradation of tissue by releasing enzymes such as cathepsin, and involved in induction of autophagy as well as survival of the cell under stressful conditions such as nutrition deficiency or fasting[6]. During cancer progression, lysosomes are upregulated to enhance the supply of certain nutrients (especially for cells that have a high energy demand) via the processes of autophagy, and then the assisting mechanisms of invasion and metastasis are also augmented via the lysosomal hydrolase and capthepsin releasing systems[7].

Various studies have shown targeting lysosome membrane channel (LMC) triggers cancer cell death but broadspectrum lysosome inhibition affects the function of normal cells as well[8,9,10]. TRPML1 is a cation channel present on late endosome and lysosome membrane. It belongs to mucolipin family of trp (Transient receptor potential channel) superfamily of ion channels, (TRPML 2 and 3 being the other two)[11]. It is widely distributed on lysosomes of different types of cells. Major role of this channel include trafficking of endolysosomes, late endosomes to lysosomes maturation, nutrient sensing and adaptation, detection of cell stress, clearance and reforming of lysosomes, phagocytosis and autophagy as well as lysosome mitochondria cross talk [11]. In cancer it modulates membrane lipids synthesis to enhance oncogenic signaling pathways[12].

TRPML1 is overexpressed in TNBC cells**,** but not in normal breast cells[13]. Due to their overexpression, they can be targeted *in vitro* and *in vivo.* It can be activated or inhibited experimentally by agonists or antagonists, respectively. Various studies showed that TRPML1 could be a useful drug target for triple-negative breast cancer[14-17]. Evidence also links the channel to widespread tumor-related events, from metabolic changes to cell migration. ML-SA5 is a small synthetic compound specifically designed to bind with TRPML1 receptor and its specificity has been confirmed by TRPML1 knockout cell lines and atomic-resolution level studies, based on the potent agonistic activity of ML-SA5, we hypothesize that treatment with low micromolar concentrations of ML-SA5 induces cytoplasmic ionic imbalance, thereby triggering rapid cell death in triple-negative breast cancer cells.

METHODOLOGY

This study was conducted in MDRL 1 and 2 Lab, Ziauddin University, Clifton Campus. The target specimen was MDA-MB-231 cell line. This is an in-vitro experimental study that was completed in 8 months after approval from GEC (Graduation Examination Committee), ERC (Reference no. 8970724 AHMM) (Ethical Review Committee) and BASR (Board of Advanced Studies and Research).

Material and Method

Trypsin-EDTA (cat. no. 25200-056), Dulbecco's Modified Eagle Medium (cat. no. 11995-065), Fetal Bovine Serum (cat. no. 10270-106) Dimethyl Sulfoxide (cat. no. 137285925), MTT dye (M6494) Phosphate Buffered Saline (10010023) Penicillin-Streptomycin (15070063). ML-SA 5 (cat. no. SML3341). Propidium iodide (cat. no. 81845). Mitotracker Red (cat. no. M7512). LysoTracker Red DND-99 (cat. no. L7528). Annexin V-FITC (cat. no. A13199). DCFH-DA dye (cat. no. D399). Hoechst 33342 (cat. no. 62249).

Cell culture

In DMEM, MDA-MB-231 breast cancer cells were cultured with 100 mg/mL penicillin-streptomycin and 10 mL of fetal bovine serum (FBS, 10%). The cultures were monitored for contamination and confluence every day while being maintained at 37 °C and 5% CO_2 in a humidified incubator. Monolayers were separated using 0.5% trypsin-EDTA after they achieved about 90% confluency. They were then either split into new flasks or used for the experiments.

MTT Cell Viability Assay

MDA-MB-231 cell lines was cultured at a density of 1 x10 4 cells per well in 96-well plates and let them rest overnight so that the cells would adhere to the surface of the incubator. Next day, cells were incubated with different concentrations of

ML-SA (0, 25, 50, 75 and 100 µM) for 1.5, 3 and 6, hours the next day. After treatment, plates were incubated for three hours after the addition of 200 µL of MTT solution. MTT was converted to purple formazan crystals by live cells and was then dissolved in 100 µL of DMSO per well. Absorbance at 570 nm was measured with a MultiSkan Sky spectrophotometer. Percent inhibitions were calculated through the following formula:

$$Cytotoxicity = \frac{OD\ of\ test - OD\ of\ blank}{OD\ of\ control - OD\ of\ blank} \times 100$$

GraphPad Prism was used to calculate IC_{50} values.

Propidium Iodide (PI) Staining Assay

MDA-MB-231 stock cells were also grown in 24-well plates at a density of 1 x 105 cells per well and kept in the incubator overnight to attach. Cells were subsequently treated for ML-SA5 at the indicated concentration for 1.5, 3 and 6 hours intervals. For 15 minutes at room temperature, cells were stained with 100 µg/mL of propidium iodide (PI) stain and left in the dark. Following staining, cells were mounted, cleaned with PBS, and examined using a Nikon Ts2-FL fluorescent microscope. Images were captured and processed through ImageJ software.

Annexin V-FITC Apoptosis Assay

In 24-well plates, MDA-MB-231 cell lines were maintained at a density of 1 x 10^5 cells per well, and they were incubated for adhesion for all of the night. Cells were treated with ML-SA5 at desired concentration for 1.5, 3 and 6, hours. After treatment, staining was performed by adding 50 µL of Annexin V-FITC to 450 µL of Annexin binding buffer and incubating for 15 minutes in the dark at room temperature. After that, the cells were rinsed with PBS, mounted, and examined using a Nikon Ts2-FL fluorescent microscope. Images were captured and processed through ImageJ software.

Cellular Reactive Oxygen Species (ROS) Assay

A black fluorescent 96-well plate was seeded with MDA-MB-231 cells (2 x 10^4) for overnight incubation. The medium was changed the next day and the cells were rinsed with PBS. 10 µM DCFH-DA dye was added for 45 min, and then ML-SA5 was added at desired concentrations. 100 µM of H_2O_2 was also added in cells as positive control. Fluorescence intesnsity was read immediately at 485/535 nm after indicated times using a microplate fluorescence reader with a gain setting optimized for the assay.

Lysotracker Red Staining

In 24 well plates, MDA-MB-231 cells were cultivated at a density of 1 × 10^5 cells per well, and they were left to incubate for the entire night. The cells were then incubated with ML-SA5 for the specified amount of time at IC_{50} the following day. After incubation, ML-SA5 was removed, 100 nM LysoTracker Red dye was added for 30 minutes at 37 °C and then rinsed three times in PBS. After being cleaned, the cells were fixed for 15 minutes in 4% paraformaldehyde. They were then rinsed with PBS and exposed to a 1:5000 diluted solution of Hoechst 33342 for 5 minutes. Images were obtained using a Nikon Ts2-FL fluorescent microscope after mounting. ImageJ software was used to process images. Images were captured and processed through ImageJ software.

MitoTracker Red Staining

1×10^5 MDA-MB-231 cells were added to each cell of 24-well plates and the cells were incubated for 1 night. Next day cells were incubated with ML-SA5 for indicated durations at IC_{50}. After that, compound was removed, 100 nM MitoTracker Red dye was added for 30 minutes at 37 °C and then rinsed three times in PBS. Following a 15-minute fixation in 4% paraformaldehyde, the cells were rinsed with PBS and subjected to a 5-minute treatment with Hoechst 33342 (1:5000 diluted). Images were obtained using a Nikon Ts2-FL fluorescent microscope after mounting. ImageJ software was used to process images. Images were captured and processed through ImageJ software.

Statistical Analysis Data

Results were evaluated using SPSS 22.0. Depending on the distribution, the continuous variables ROS levels, cell viability, and cell death were summarized as mean ± SD or median [IQR]. Numbers and percentages were used to represent categorical results, such as positive LysoTracker Red and MitoTracker Red staining. One-way ANOVA and post-hoc comparisons were used for comparisons involving continuous data. For categorical data, a chi-square analysis was performed. The threshold for statistical significance was set at a p value of 0.05.

RESULTS

MTT Assay

Cells were exposed to a range of compound doses for 1.5, 3, or 6 hours duration, % inhibitions were calculated and IC_{50} was determined. It was observed that ML-SA5 drastically decreased cell viability and caused cytotoxicity at even lover concentrations of drug. Overall cell survival decreased in a dose-dependent manner as shown in figure-1. IC_{50} of ML-SA5 against MDA-MB-231 was found to be 6.8, 11.5 and 12.3 µM at 1.5, 3 and 6 hours respectively.

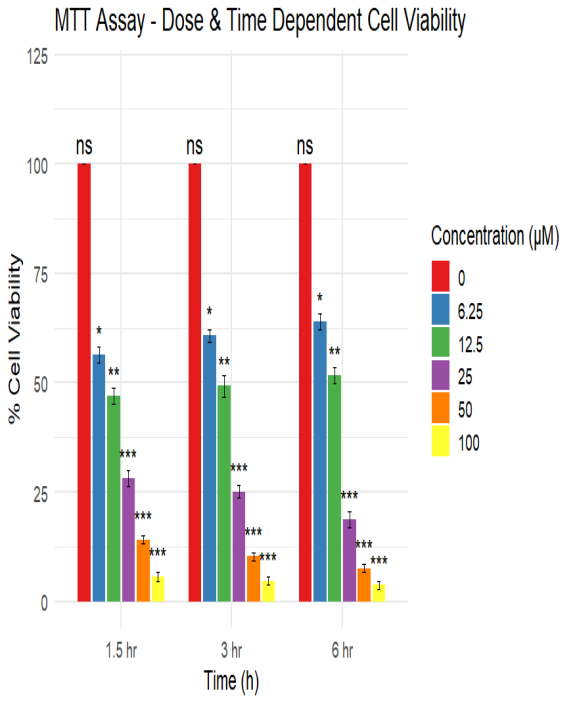

Figure 1: Viability of cells after 1.5, 3, and 6 hours of treatment, as assessed by the MTT assay. Results are the mean ± SD of three independent tests. *p<0.05

PI Staining Assay

To further confirm cell death, PI staining was performed on MDA-MB-231 cells treated with ML-SA5 at concentrations above and below the IC_{50} (3.15, 6.25, 12.5, and 25 µM) for 1.5 h, 3 h, and 6 h. A dose-dependent increase in PI-positive cells was observed, indicating cell death upon TRPML1 activation. While longer treatment slightly enhanced cell death, the difference across time points was minimal. Minimal PI staining was observed in control cells. Overlay phase contrast images confirmed morphological changes and increased PI uptake in treated cells (Figure 2A-B).

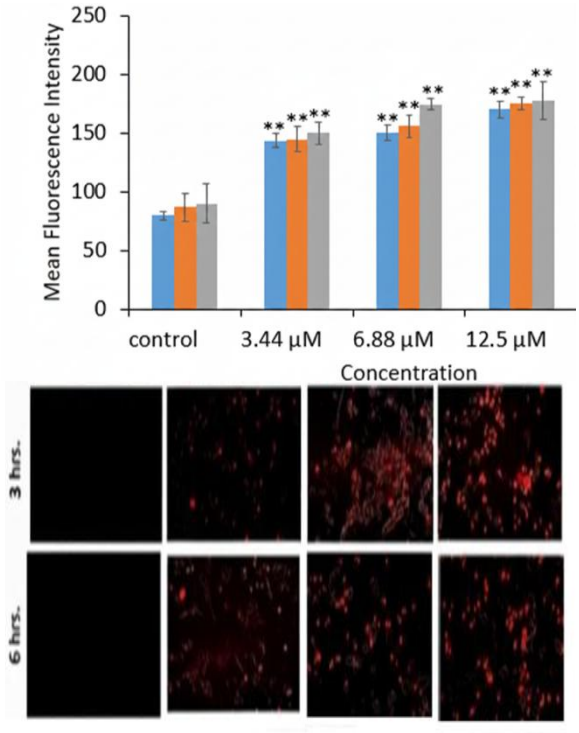

Figure 2: Assessment of compound-induced cell death via PI staining at various concentrations and time points. (A) Representative fluorescence microscopy images of cells stained with propidium iodide (PI). Red fluorescence indicates PI-positive (non-viable) cells (B) Quantification of PI staining represented as mean fluorescence intensity for each concentration and time point. Data are presented as mean ± SD. Statistical significance compared to control: **p < 0.01.

Annexin V Assay

To further validate the cell death, annexin V was used to evaluate apoptosis. Since optimal cell death was observed at 1.5h in both MTT And PI assays, therefore, this time was selected for further experiments. At 1.5 hr, considerable apoptosis was observed in treated group as compared to control at IC50 concentration.

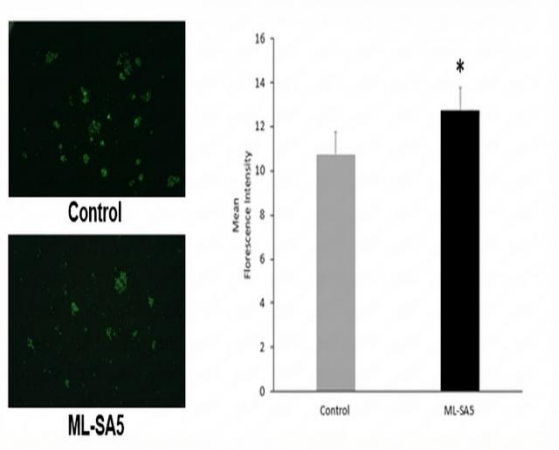

Figure 3: Annexin V-FITC labelling was used to evaluate the ML-SA5's ability to induce apoptosis at IC_{50} after 1.5 hours. Results are the mean ± SD of three independent tests. Student's t-test was used to assess statistical significance (p < 0.01 compared to control).

MitoTracker Red staining

Cells were stained with MitoTracker Red and then examined with microscopy for analyzing mitochondrial morphology. The pictures demonstrated that ML-SA5-induced TRPML1 activation resulted in a morphological change that raised the intensity of the florescence (Figure 4). These findings showed that altering TRPML1 activity by activation, significantly changes mitochondrial morphology. Results are the mean ± SD of three independent tests. Student's t-test was used to assess statistical significance (p < 0.01 compared to control).

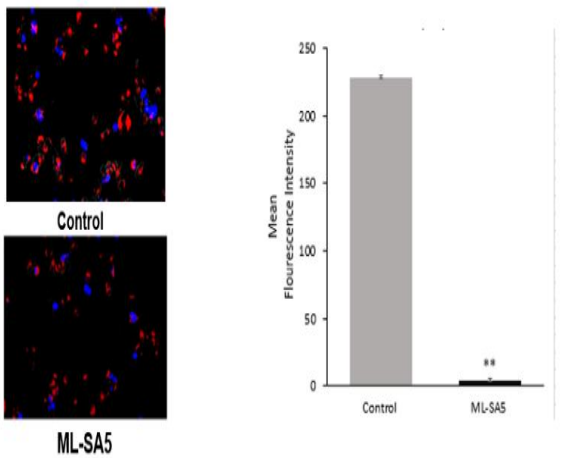

Figure 4: Mitotracker red (mitochondria) and Hoechst 33342 (nuclei) were used to evaluate the mitochondrial morphology of treated with ML-SA5(IC_{50}) and intensity was measured by Image J.

Lysotracker Red staining

Lysosomes were stained with Lysotracker dye after treatment with ML-SA5 and images were captured. It was found that ML-SA5 increased lysosomal activity as indicated by Lysotracker Red intensity in treated group. It may indicate cell stress or induction of autophagy in response to the treatment with ML-SA5.

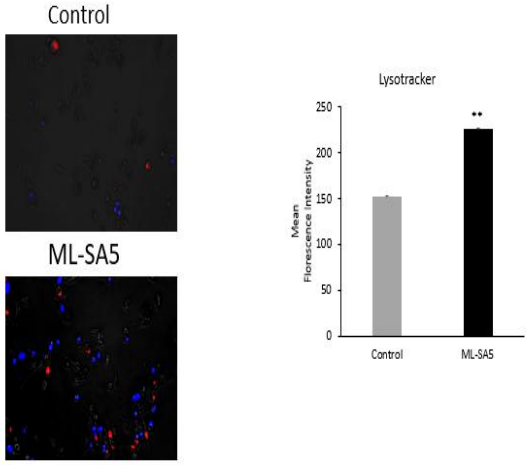

Figure 3 Morphology of lysosomes was observed by LysoTracker Red staining and Hoechst 33342 (nuclei) and lysosomal staining intensity was measured by Image J (n=30).

ROS Assay

Excessive intracellular ROS generation indicates cell death due to ion imbalance homeostasis triggered on by dysregulation of the lysosome and mitochondria. We used DCFH-DA to detect intracellular ROS levels after treatment with ML-SA5. The results showed that activating TRPML1 causes significant increase in the levels of ROS. Cells were treated with ML-SA at concentrations of 3.5 µM and 25 µM for the indicated duration, and ROS production was measured using DCFH-DA staining. H_2O_2 (100 µM) was used as a positive control. Fluorescence intensity of DCF was measured and plotted as mean ± SD from three independent experiments. (*p < 0.01).

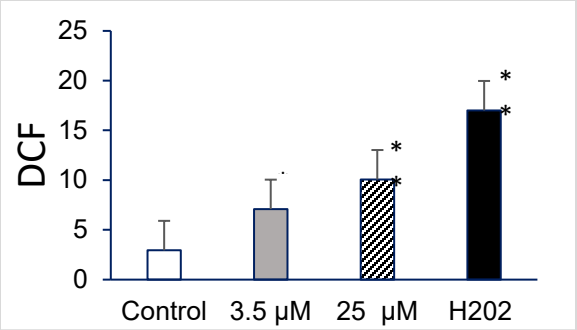

Figure 6: ML-SA treatment induces intracellular ROS generation in MDA-MB-231 cells.

DISCUSSION

In the current work, we examined the possible therapeutic application of ML-SA5, a selective TRPML1 agonist, to cause cell death of MDA-MB-231 triple-negative breast cancer (TNBC) cell line.

Poor prognosis, invasion, migration, and tumor proliferation are all positively connected with TRPML1 overexpression [18]. Our findings collectively highlight that TRPML1 activation triggers a cascade of events involving mitochondrial and lysosomal dysfunction, ultimately leading to cancer cell death. According to recent research, TRPML1 dysfunction inhibits different cancer cells' ability to migrate in vitro and decreases the spread of cancer in vivo [18].

TRPML1 may be a promising target for anti-tumor therapy, as our study also revealed that pharmacological activation can inhibit the progression of cancer. The MTT assay was initially used to demonstrate the cytotoxic effect of ML-SA5, showing dose-dependent decreases in cell viability at different time durations (1.5, 3, and 6 hours), with IC_{50} values in the low micromolar range. There may be some specificity for malignant cells because these values were noticeably greater in cancer cells than in non-malignant HUVECs. ML-SA5 is a viable option for short-exposure therapies because of its relatively early cytotoxic effects, as seen by cell death at lower concentration.

With TRPML1's established function in controlling lysosomal and mitochondrial homeostasis, we evaluated at the subcellular effects of ML-SA5 treatment. MitoTracker staining showed increased fluorescence intensity and changed mitochondrial shape. These structural alterations probably affect mitochondrial function and play a role in cell death and energy failure. Larger lysosomal structures were shown by LysoTracker labeling. These findings imply that ML-SA5 disrupts lysosomal integrity, which is in line with the effects of TRPML1 activation that are well-established and with other findings that associate lysosomal dysfunction with compromised autophagic flux and necrotic cell death pathways [19].

Propidium iodide (PI) staining experiments were used to validate the cell death. In a dose-dependent manner, ML-SA5 therapy increased the number of PI-positive cells, which is a sign of membrane-compromised necrotic cell death. This was corroborated by annexin V assays, which showed considerable cell death in viability tests but only a small amount of apoptosis at the 1.5-hour treatment point. These findings imply that ML-SA5 causes MDA-MB-231 cells to undergo cell death.

The significance of the lysosomal TRPML1 channel for mitochondria has been extensively studied since it is a crucial regulator of ion homeostasis [20]. Cancer cells have a high energy requirement; thus, lysosome function is typically elevated. It assists in tumor progression by releasing hydrolase and cathepsin enzymes to facilitate invasion and metastasis and by delivering nutrition through autophagy. Various studies have shown that targeting LMC (lysosome membrane channels) triggers cancer cell death. In cancer, it modulates membrane lipid synthesis to enhance oncogenic signaling pathways. Due to their overexpression, they can be targeted *in vitro* and *in vivo.* It can be activated or inhibited experimentally by agonists or antagonists, respectively. According to studies, the inhibition or knockdown of TRPML1 may result in reduced proliferation of cancer cells[16,21]. On the same note, blockage of TRPML1 with highly potent ML-SIs could barely block the proliferation of several cancer cell lines[22]. Associated with these findings, small-molecule activation of TRPML1, rather than inhibition is more desirable to induce selective cancer cell death in vitro and in vivo.

The modulation of mitochondrial structure, membrane potential, and ROS formation is a critical function of ions such as calcium, zinc, and iron. Modifying calcium-permeable TRPML1 may have an impact on mitochondrial function,

considering the significance of ions in mitochondrial control [23]. According to our results, the stimulation of TRPML1 through ML-SA5 increased the levels of mitochondrial and cytosolic ions. The phenotype supports the earlier discovery, which showed that mitochondria enhance ATP generation by increasing the absorption of excess calcium from the cytosol [24]. On the other hand, mitochondria experience ion overload. Uncontrollable mitochondrial calcium leads to overproduction of ROS, permeability transition pore (mPTP) opening, and cytochrome c release that eventually leads to cell death [25]. In line with our results, we found that the mitochondrial membrane became disoriented with the activation of TRPML1 (Figure 4). This was most likely caused by the opening of mPTP because cyclosporin A, a known blocker of transition pore development, could restore the membrane potential[29].

An important mechanistic insight was provided by the ROS assay, which demonstrated a significant increase in intracellular ROS levels after ML-SA5 treatment. The observed oxidative stress is compatible with defective autophagy, mitochondrial dysfunction, and TRPML1 activation-induced disruption of ion homeostasis. ML-SA5 promotes necrotic cell death through multiple converging stress pathways is further supported by elevated ROS levels, which are known mediators of cell death.

Our observation that TRPML1 stimulation raises mitochondrial reactive oxygen species (Figure 6) and consequently diminishes the organelles' ATP production is consistent with evidence that excess mitochondrial ROS inflicts oxidative damage and impairs ATP synthesis [25]. We postulate that cytosolic Ca2+ overload activates calcium-induced calcium release (CICR), further amplifying the signal, since TRPML1 hyperactivity already elevates global calcium levels. In line with this, TRPML1-mediated calcium efflux can trigger the endoplasmic reticulum (ER) to release additional Ca2+ via inositol trisphosphate (IP3) and ryanodine receptors (RyR) [26]. Increased cytosolic calcium, in turn, enters mitochondria through the mitochondrial calcium uniporter (MCU) and channels such as VDAC, intensifying oxidative stress [27]. When mitochondrial Ca2+ surges exceed a tipping point, they compromise membrane stability, depolarize the inner membrane potential, elevate ROS production, and push the cell toward apoptosis or necrosis.

Supporting this perspective, more recent studies show that the lysosomal TRPML1 channel controls calcium movement at the contact points between lysosomes and mitochondria, marking it as a major gatekeeper of that inflow [28]. Because TRPML1 is a cation channel anchored in the lysosomal membrane, its opening can also raise the organelles Zn2+; high Zn2+ levels have been associated with necrotic like cell death in melanoma cells [21].

CONCLUSION

Selective agonist and TRPML1-ML-SA5 were found to induce cell death in the triple-negative breast cancer (TNBC) cell line MDA-MB-231 in a time and dose-dependent fashion. Subsequent mechanistic investigations demonstrated that ML-SA5 treatment led to elevated reactive oxygen species (ROS) production and disrupted both lysosomal integrity and mitochondrial morphology. These findings suggest that ML-SA5exerts cytotoxic effects via oxidative stress and organelle destabilization pathways. Taken together, these findings indicate that ML-SA5 can be a possible therapeutic target in the treatment of TNBC.

Ethical Approval

The study received a waiver of ethical approval from the Ethical Review Committee of Ziauddin University (Reference Code: 8970724AHMM).

Author Contributions

AH: Data Collection, Data Analysis & Interpretation, Manuscript Writing, Critical Revision
RI: Conception & Design, Data Collection Data Analysis & Interpretation, Manuscript Writing, Critical Revision
SA: Data Analysis & Interpretation, Manuscript Writing
SSR: Data Collection, Manuscript Writing
AB: Data Collection, Manuscript Writing
SAK: Conception & Design
All authors approved the final version of the manuscript to be published.

Grant Support and Funding Disclosure

None.

Conflict of Interests

No conflict of interest.

REFERENCES

1. Wilkinson L, Gathani T. Understanding breast cancer as a global health concern. Br J Radiol [Internet]. 2022;95(1130):20211033. Available from: http://dx.doi.org/10.1259/bjr.20211033
2. Zhao H. The prognosis of invasive ductal carcinoma, lobular carcinoma and mixed ductal and lobular carcinoma according to molecular subtypes of the breast. Breast Cancer [Internet]. 2021;28(1):187–95. Available from: http://dx.doi.org/10.1007/s12282-020-01146-4
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin [Internet]. 2021;71(3):209–49. Available from: http://dx.doi.org/10.3322/caac.21660
4. Al-Thoubaity FK. Molecular classification of breast cancer: A retrospective cohort study. Ann Med Surg (Lond) [Internet]. 2020;49:44–8. Available from: http://dx.doi.org/10.1016/j.amsu.2019.11.021
5. Baranova A, Krasnoselskyi M, Starikov V, Kartashov S, Zhulkevych I, Vlasenko V, et al. Triple-negative breast cancer: current treatment strategies and factors of negative prognosis. J Med Life [Internet]. 2022;15(2):153–61. Available from: http://dx.doi.org/10.25122/jml-2021-0108
6. Zhao W, Ren W, Huang D, Sang Y, Cao L, Huang J. Cell structure and physiology. In: Cell Movement in Health and Disease. Elsevier; 2022. p. 3–16.
7. Tang T, Yang Z-Y, Wang D, Yang X-Y, Wang J, Li L, et al. The role of lysosomes in cancer development and progression. Cell Biosci [Internet]. 2020;10(1):131. Available from: http://dx.doi.org/10.1186/s13578-020-00489-x
8. Feng J, Wang Z-X, Bin J-L, Chen Y-X, Ma J, Deng J-H, et al. Pharmacological approaches for targeting lysosomes to induce ferroptotic cell death in cancer. Cancer Lett [Internet]. 2024;587(216728):216728. Available from: http://dx.doi.org/10.1016/j.canlet.2024.216728
9. Reisbeck L, Linder B, Tascher G, Bozkurt S, Weber KJ, Herold-Mende C, et al. The iron chelator and OXPHOS inhibitor VLX600 induces mitophagy and an autophagy-dependent type of cell death in glioblastoma cells. Am J Physiol Cell Physiol [Internet]. 2023;325(6):C1451–69. Available from: http://dx.doi.org/10.1152/ajpcell.00293.2023
10. Serrano-Puebla A, Boya P. Lysosomal membrane permeabilization as a cell death mechanism in cancer cells. Biochem Soc Trans [Internet]. 2018;46(2):207–15. Available from: http://dx.doi.org/10.1042/BST20170130
11. Santoni G, Morelli MB, Amantini C, Nabissi M, Santoni M, Santoni A. Involvement of the TRPML mucolipin channels in viral infections and anti-viral innate immune responses. Front Immunol [Internet]. 2020;11:739. Available from: http://dx.doi.org/10.3389/fimmu.2020.00739
12. Xu M, Dong RY, Wang P, Dai E, Dong X-P. Physiological and pathological functions of TRPML1. In: TRP-Mediated Signaling. Boca Raton: CRC Press; 2024. p. 107–30.
13. Pan Y, Zhao Q, He H, Qi Y, Bai Y, Zhao J, et al. TRPML1 as a potential therapeutic target for triple-negative breast cancer: a review. Front Oncol [Internet]. 2023;13:1326023. Available from: http://dx.doi.org/10.3389/fonc.2023.1326023
14. Liu HY, Gale JR, Reynolds IJ, Weiss JH, Aizenman E. The multifaceted roles of zinc in neuronal mitochondrial dysfunction. Biomedicines [Internet]. 2021;9(5):489. Available from: http://dx.doi.org/10.3390/biomedicines9050489
15. Qi J, Xing Y, Liu Y, Wang M-M, Wei X, Sui Z, et al. MCOLN1/TRPML1 finely controls oncogenic autophagy in cancer by mediating zinc influx. Autophagy [Internet]. 2021;17(12):4401–22. Available from: http://dx.doi.org/10.1080/15548627.2021.1917132
16. Almasi S, Kennedy BE, Yoast RE, Emrich SM, Trebak M, Hiani YE. The lysosomal TRPML1 channel promotes breast cancer survival by supporting mitochondrial function and cellular metabolism [Internet]. bioRxiv. 2020. Available from: http://dx.doi.org/10.1101/2020.09.04.283242
17. Wang W, Gao Q, Yang M, Zhang X, Yu L, Lawas M, et al. Up-regulation of lysosomal TRPML1 channels is essential for lysosomal adaptation to nutrient starvation. Proc Natl Acad Sci U S A [Internet]. 2015;112(11):E1373-81. Available from: http://dx.doi.org/10.1073/pnas.1419669112
18. Marini M, Titiz M, Souza Monteiro de Araújo D, Geppetti P, Nassini R, De Logu F. TRP channels in cancer: Signaling mechanisms and translational approaches. Biomolecules [Internet]. 2023;13(10):1557. Available from: http://dx.doi.org/10.3390/biom13101557
19. Siow WX, Kabiri Y, Tang R, Chao Y-K, Plesch E, Eberhagen C, et al. Lysosomal TRPML1 regulates mitochondrial function in hepatocellular carcinoma cells. J Cell Sci [Internet]. 2022;135(6). Available from: http://dx.doi.org/10.1242/jcs.259455
20. Kendall RL, Holian A. The role of lysosomal ion channels in lysosome dysfunction. Inhal Toxicol [Internet]. 2021;33(2):41–54. Available from: http://dx.doi.org/10.1080/08958378.2021.1876188
21. Xu M, Almasi S, Yang Y, Yan C, Sterea AM, Rizvi Syeda AK, et al. The lysosomal TRPML1 channel regulates triple negative breast cancer development by promoting mTORC1 and purinergic signaling pathways. Cell Calcium [Internet]. 2019;79:80–8. Available from: http://dx.doi.org/10.1016/j.ceca.2019.02.010
22. Du W, Gu M, Hu M, Pinchi P, Chen W, Ryan M, et al. Lysosomal Zn2+ release triggers rapid, mitochondria-mediated, non-apoptotic cell death in metastatic melanoma. Cell Rep [Internet]. 2021;37(3):109848. Available from: http://dx.doi.org/10.1016/j.celrep.2021.109848
23. Peng W, Wong YC, Krainc D. Mitochondria-lysosome contacts regulate mitochondrial Ca2+ dynamics via lysosomal TRPML1. Proc Natl Acad Sci U S A [Internet]. 2020;117(32):19266–75. Available from: http://dx.doi.org/10.1073/pnas.2003236117
24. Feng X, Cai W, Li Q, Zhao L, Meng Y, Xu H. Activation of lysosomal Ca2+ channels mitigates mitochondrial damage and oxidative stress. J Cell Biol [Internet]. 2025;224(1). Available from: http://dx.doi.org/10.1083/jcb.202403104
25. Morciano G, Naumova N, Koprowski P, Valente S, Sardão VA, Potes Y, et al. The mitochondrial permeability transition pore: an evolving concept critical for cell life and death. Biol Rev Camb Philos Soc

[Internet]. 2021;96(6):2489–521. Available from: http://dx.doi.org/10.1111/brv.12764
26. Zhang H-L, Hu B-X, Ye Z-P, Li Z-L, Liu S, Zhong W-Q, et al. TRPML1 triggers ferroptosis defense and is a potential therapeutic target in AKT-hyperactivated cancer. Sci Transl Med [Internet]. 2024;16(753):eadk0330. Available from: http://dx.doi.org/10.1126/scitranslmed.adk0330
27. Nasb M, Kirberger M, Chen N. Molecular processes and regulation of autophagy. In: Exercise, Autophagy and Chronic Diseases. Singapore: Springer Singapore; 2021. p. 1–27.
28. Rosencrans WM, Rajendran M, Bezrukov SM, Rostovtseva TK. VDAC regulation of mitochondrial calcium flux: From channel biophysics to disease. Cell Calcium [Internet]. 2021;94(102356):102356. Available from: http://dx.doi.org/10.1016/j.ceca.2021.102356
29. Mdpi.com. [cited 2025 Dec 24]. Available from: https://www.mdpi.com/2073-4409/10/1/125