Lipids in Health and Disease

RESEARCH

Open Access

The protumorigenic enzyme GPAT2 inhibits arachidonic acid-triggered apoptosis in breast cancer

Fiorella J. Ferremi[1†], Veronica V. Moscoso[1†], Mauro A. Montanaro[1], Maria R. Gonzalez-Baro[1] and Elizabeth R. Cattaneo[1*]

Abstract

Background Cancer is a significant health challenge and the leading cause of mortality globally. Tumor cells use multiple mechanisms to acquire their distinctive capacity for uncontrolled proliferation, one of which is the evasion of apoptosis. It has been shown that in breast, colon, and liver cancer, evasion of apoptosis is associated with the overexpression of enzymes that metabolize arachidonic acid (AA) because free AA is a strong inducer of apoptosis. Glycerol–3-phosphate acyltransferase 2 (GPAT2) is a key enzyme in AA metabolism and is highly expressed in breast and colon cancer, where it promotes the development of essential tumor features.

Methods In this work, a model of GPAT2 silencing in the human breast cancer-derived cell line MDA-MB-231 was used, and the cells were exposed to exogenous AA. The role of GPAT2 in AA-induced cell death was studied using MTT and TUNEL assays and measurements of caspase activity. The underlying molecular mechanism of cell death was assessed by qRT–PCR.

Results The results showed that AA reduced cell viability only in GPAT2-silenced cells, and that this cell death was a consequence of an apoptotic process involving BNIP3 overexpression. Additionally, it was demonstrated that GPAT2 silencing triggered a compensatory mechanism by overexpressing other genes involved in AA utilization for eicosanoid biosynthesis.

Conclusions We concluded that GPAT2 expression is necessary to prevent AA-induced apoptotic cell death in MDA-MB-231 cells and that the overexpression of other AA-metabolizing genes is not sufficient to compensate for the lack of GPAT2 and prevent apoptosis.

Keywords GPAT2, Arachidonic acid, Apoptosis, Breast cancer, Cell viability, BNIP3

[†]Fiorella J. Ferremi and Veronica V. Moscoso contributed equally to this work.

*Correspondence:
Elizabeth R. Cattaneo
ecattaneo@med.unlp.edu.ar
[1]Instituto de Investigaciones Bioquímicas de La Plata Dr. Rodolfo R. Brenner, Consejo Nacional de Investigaciones Científicas y Técnicas, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina

Introduction

The World Health Organization reports that breast cancer is the most common cancer worldwide and the primary cause of cancer-related deaths among women. Prevention and early diagnosis are recognized as the most effective strategies for cancer control. Consequently, the study of new tumor markers has gained significant attention. In our laboratory, we demonstrated that GPAT2 is highly expressed in several types of cancer, including melanoma, lung, prostate and undifferentiated breast carcinomas [1].

Glycerol–3-phosphate acyltransferase (GPAT) enzymes catalyze the first step in the de novoglycerolipid synthesis pathway, the acylation of glycerol–3-phosphate by long-chain fatty acyl-CoA. Four isoforms of GPATs are known. GPAT1, 3 and 4 are expressed in lipogenic organs and use saturated or monounsaturated acyl-CoAs as substrates. GPAT2 is detected in normal male germline cells and in several cancers and is the only isoform that uses the polyunsaturated ω-6 fatty acid arachidonic acid (AA) as a substrate [1, 2]. Due to its particular expression pattern GPAT2 has been classified as a member of the 'cancer-testis genes', a group of genes primarily expressed in spermatogenic cells, with minimal or no expression in normal somatic tissues. At protein level GPAT2 is expressed in approximately 37% of human breast adenocarcinomas, and we have already shown that cancer cells with reduced GPAT2 expression show decreased proliferation, migration, and tumorigenic potential in mouse xenograft models, as well as an increased sensitivity to staurosporine-induced apoptosis [1].

AA and the eicosanoids generated as a result of AA metabolism play crucial roles in cancer development. Intracellular free AA is a potent inducer of apoptosis, and metabolic pathways that decrease AA serve as preventive mechanisms against cell death [3-6]. For this reason, drugs [7] and natural products that inhibit the AA pathway have been proposed as therapeutic agents for various cancer types [8].

Cellular AA can be metabolized through two pathways. The first involves the synthesis of eicosanoids, a process catalyzed by enzymes belonging to the cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 enzyme families. The second pathway leads to glycerolipid synthesis and involves enzymes from the long-chain acyl-CoA synthetases (ACSL) family, GPAT2 and AGPAT isoforms, and phospholipid remodeling enzymes [9].

One of the key characteristics of tumor cells is their ability to evade apoptosis, which is one of the hallmarks of cancer [10]. Studies investigating the correlation between these enzymes and cancer have revealed the overexpression of COX and LOX enzymes in various tumors, and researchers have been exploring inhibitors of these pathways for their potential antitumor effects [6, 11]. Furthermore, overexpression of ACSL4 (an isoform specifically involved in AA activation) has been observed in breast and colon cancer and is related to the protection of tumor cells from AA-induced apoptosis. [6, 12, 13]. These findings underscore the importance of regulating intracellular free AA levels for cancer cell survival. Given the involvement of GPAT2 in AA metabolism and its particular pattern of expression, exploring the role of GPAT2 in tumor cells is crucial for comprehending the molecular mechanisms that connect AA to the tumorigenic process.

This work aimed to investigate the impact of GPAT2 on cell viability and apoptosis activation pathways triggered by AA treatment. We hypothesize that GPAT2 expression in breast cancer cells is essential for preventing apoptosis induced by AA. Specifically, we propose that silencing GPAT2 will lead to increased AA-induced apoptosis through the upregulation of proapoptotic factors highlighting the critical role of GPAT2 in maintaining cancer cell viability and contributing to tumorigenic processes in breast cancer.

Methods

Material and reagents

All the chemicals were purchased from Sigma unless otherwise indicated.

Cell lines and culture conditions

Human breast adenocarcinoma MDA-MB–231 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The MDA-MB-231 cell line was selected because of its high GPAT2 expression. Cell lines stably expressing a small hairpin RNA targeting GPAT2 mRNA (shRNA-GPAT2) (HuSH–29 plasmid, OriGene) or a non-silencing scrambled RNA (shRNA-scr) were generated in our laboratory, as we previously reported [1], to generate sh-MDA (reduced GPAT2 expression) and scr-MDA (retaining GPAT2 expression) cell lines. The cells were grown at 37 °C in a 5% CO2 atmosphere with 98% relative humidity and were routinely cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% FBS (Natocor, Argentina), 100 U/ml penicillin and 100 µg/ml streptomycin. GPAT2 knockdown was routinely assessed by quantitative real-time PCR (qRT–PCR) with the most recent validation shown in Fig. 1 of the Supplementary Material.

Quantitative real-time PCR

Total RNA was isolated from cell lines using TRIzol (Life Technologies) following the manufacturer's instructions, and RNA quality was determined by gel electrophoresis and 260/230 and 260/280 nm OD ratios. One microgram of RNA was used for cDNA synthesis via an

iScript cDNA synthesis Kit (Bio-Rad). A 1:2 cDNA dilution was used for qRT−PCR with iTaq Universal Sybr Green SuperMix (Bio-Rad). The following primers were designed for amplification:
AKR1C3
forward primer: GTACAAGCCTGTCTGCAACC.
reverse primer: AGACAAACGATGGGTGGACC.
ALOX5
forward primer: GGATGGACGCGCAAAGTTGG.
reverse primer: CCTTGTGGCATTTGGCATCG.
EPHX2
forward primer: TGCCCAGAGGACTTCTGAATG.
reverse primer: GCAGACTTTAGCGGTCTCGG.
PTGS2
forward primer: CGGTGAAACTCTGGCTAGACAG.
reverse primer: GCAAACCGTAGATGCTCAGGGA.
BNIP3
forward primer: CAGGGCTCCTGGGTAGAACT.
reverse primer: CTACTCCGTCCAGACTCATGC.
BNIP3L
forward primer: TTCTCACTGTGACAGCCCTT.
reverse primer: AGTCTGATACCCAGTCCGCA.
TNFRSF21
forward primer: GTGACTCTACATCCAGCGGC.
reverse primer: TCTTCAATCACCCGCAGCTC.

The thermal profile was 95 °C for 1 min, followed by 40 cycles of 95 °C for 20 s, 61 °C for 50 s and 60 °C for 30 s on a Stratagene Mx3000P apparatus. RNA expression was quantified in triplicate using the ΔΔCt method and normalized to that of TBP or B-actin housekeeping genes using Qbase software.

Arachidonic acid treatment

AA stock solution (50 mM) was prepared using AA (Sigma sodium salt) complexed with fatty acid-free BSA at a 2:1 ratio and diluted with a routine medium at the final concentration indicated in each experiment. A control was generated using fatty acid-free BSA diluted in PBS under the same conditions. The culture medium was changed every day.

Cell proliferation assay

Cell proliferation was assessed by reducing the MTT (3-4,5-dimethylthiazol−2-yl)−2,5-diphenyltetrazolium bromide) reagent [14]. Fifteen thousand scr-MDA or sh-MDA cells were seeded in 48-well plates and cultured in routine medium supplemented with 50 or 100 µM AA (Sigma sodium salt) or in PBS (control) for 24–48 h. Viability was measured by adding 200 µL of 1/10 MTT stock solution (5 mg/ml in PBS, pH 7.5) to each well at the indicated time points and incubating for 4 h at 37 °C in the dark. Then, 250 µL of solubilizing solution (0.04 M HCl in isopropanol) was added to each well, and the mixture was incubated for 20 min at room temperature. Finally, the absorbance of the plates was read at 560 nm and 640 nm for background subtraction using a Beckman Coulter Multimode microplate reader (DTX−880). Six wells per condition in three independent experiments were analyzed.

Apoptosis detection TUNEL assay

Apoptotic DNA fragmentation was detected using a TUNEL assay. Briefly, sh-MDA and scr-MDA cells were seeded in triplicate on coverslips, placed in 6-well plates and allowed to grow in a routine medium until the cell density reached 70% confluence. Then, the cells were treated with 100 µM AA (Sigma, sodium salt) or PBS (control) for 48 h. A terminal deoxynucleotidyl transferase-mediated dUTP (29-deoxyuridine 59-triphosphate)-digoxigenin nick end labeling (TUNEL) assay was performed on cultured cells using an In Situ Cell Death Detection Kit (Roche) according to the manufacturer's instructions. Finally, the coverslips were mounted on slides and stained with hematoxylin. The percentage of apoptotic cells was determined by counting the number of apoptotic (TUNEL-positive) and nonapoptotic cells across thirty 40X randomly selected fields using an optical microscope (Nikon, E100).

Caspase activity assay

Caspase activity in cell culture was measured using the Caspase Substrate Colorimetric Kit (Abcam ab102486). sh-MDA cells were grown in 100 mm plates in a routine medium supplemented with 100 µM AA (Sigma sodium salt) or PBS (control) for 48 h, after which caspase 1, 2, 3, 5, 6, 8, and 9 activities were measured via a Beckman Coulter Multimode microplate reader (DTX−880) according to the manufacturer's instructions at 405 nm. Staurosporine (1 µM, 0.01% final concentration of DMSO) was used as a positive control for caspase-3 activity and was added to the culture medium for 16 h. Three independent experiments were analyzed.

Statistical analysis

Differences between the control and silenced cells were analyzed by Student's t-test or ANOVA test. Results were considered significant at the 5% level. All the statistical analysis and the graphs presented were done using GraphPad Prism software ((Windows version 7.01).

Results

Arachidonic acid reduces cell viability and triggers apoptosis exclusively in cells in which *GPAT2* is silenced

The regulation of cellular AA levels plays a critical role in the survival and proliferation of mammalian cells. This is because elevated levels of AA increase apoptosis, whereas metabolic pathways that decrease the concentration of unesterified AA have the potential to inhibit this

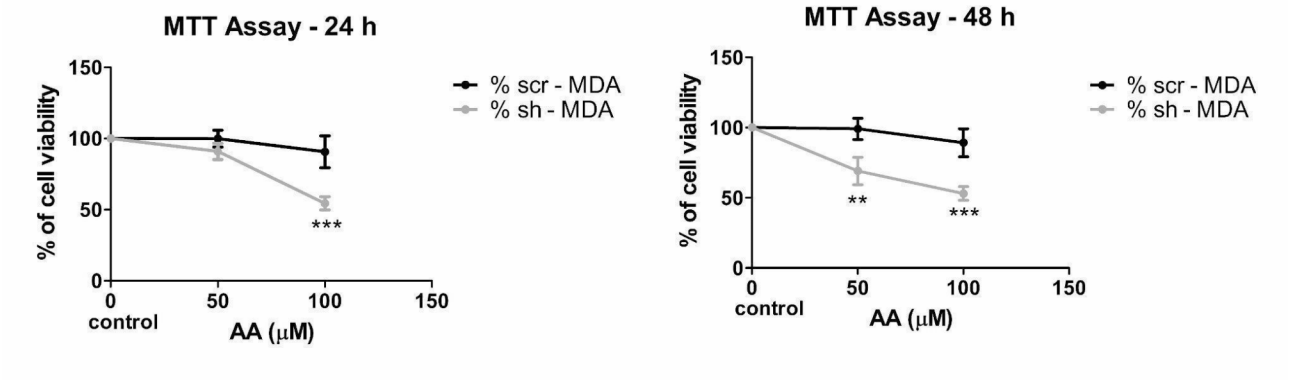

Fig. 1 Concentration-dependent induction of AA-related cell death in GPAT2-silenced cells. scr-MDA and sh-MDA cells were grown in 10% FBS DMEM supplemented with 50 or 100 μM AA for 24–48 h before the cell proliferation rate was measured via an MTT proliferation assay. The values represent the means ± SDs of 3 independent experiments (***$P < 0.001$, **$P < 0.01$)

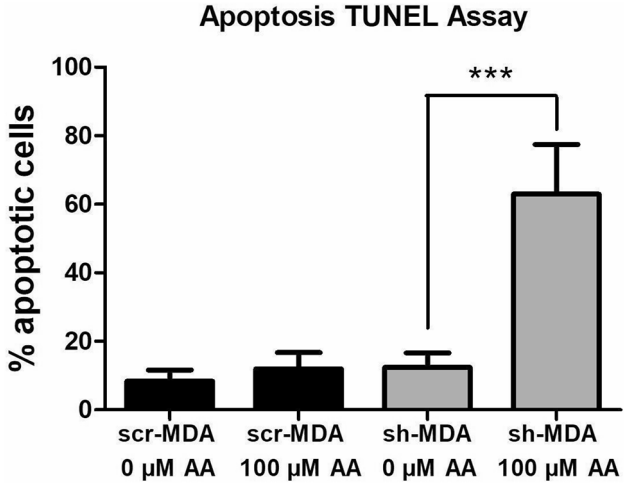

Fig. 2 AA induces apoptosis only in GPAT2-silenced cells. scr-MDA and sh-MDA cells were treated with 100 μM AA for 48 h, and the percentage of apoptotic cells was determined by counting the number of apoptotic and nonapoptotic cells via a TUNEL assay and hematoxylin staining (***$P < 0.001$)

process. To determine the role of GPAT2 in AA-induced cell death, scr-MDA and sh-MDA cells were treated with 50 or 100 μM AA for 24–48 h, and cell viability was assessed using the MTT assay. In a concentration-dependent manner, AA significantly decreased cell survival only in the GPAT2-knockdown cells (sh-MDA; Fig. 1), in which the percentage of viable cells decreased to 52.9% after incubation with 100 μM AA for 48 h.

To determine whether sh-MDA cell death occurred through apoptosis both, scr-MDA and sh-MDA cells were treated with 100 μM AA for 48 h and the percentage of apoptotic cells was quantified using a TUNEL assay (Fig. 2). The results showed that in sh-MDA cells, the percentage of apoptotic cells increased 4-fold after AA treatment, whereas no difference was found in the percentage of *GPAT2*-expressing cells (scr-MDA).

Considering that AA triggers apoptosis in MDA-MB–231 cells only in the absence of GPAT2, we questioned which apoptotic pathway was activated by AA in sh-MDA cells and whether other enzymes able to convert AA into proapoptotic products were expressed in sh-MDA cells.

In sh-MDA cells, AA-induced apoptosis involves *BNIP3* overexpression

Caspase activation plays a central role in the execution of apoptotic cell death, and the TUNEL assay determined that only *GPAT2*-silenced cells were affected by AA. To evaluate the apoptotic pathway, sh-MDA cells were treated with 100 μM AA for 48 h and caspase activities were measured using a colorimetric assay. The results showed that AA significantly increased the activity of all caspases evaluated (Fig. 3), including those related to apoptosis (caspases 2, 8, 9, 3, and 6) and those involved in inflammatory processes (caspases 1 and 5). Considering the results for caspase–3, AA exerted a more potent effect than staurosporine.

It has already been reported that AA can promote apoptosis in cancer models through a number of different mechanisms, such as activating caspases and other proapoptotic enzymes, increasing the production of potentially cytotoxic lipid peroxides and interacting with proapoptotic proteins of the Bcl–2 family, and altering mitochondrial permeability [3, 15]. In particular, the expression levels of the proapoptotic Bcl–2 family member *BNIP3*, its functional homolog *BNIP3L* and the cell death surface receptor *TNFRSF21* were assessed. Treatment of sh-MDA cells with 100 μM AA for 48 h significantly increased only *BNIP3*mRNA levels, which is consistent with our previous finding that AA induces apoptosis in these cells (Fig. 4).

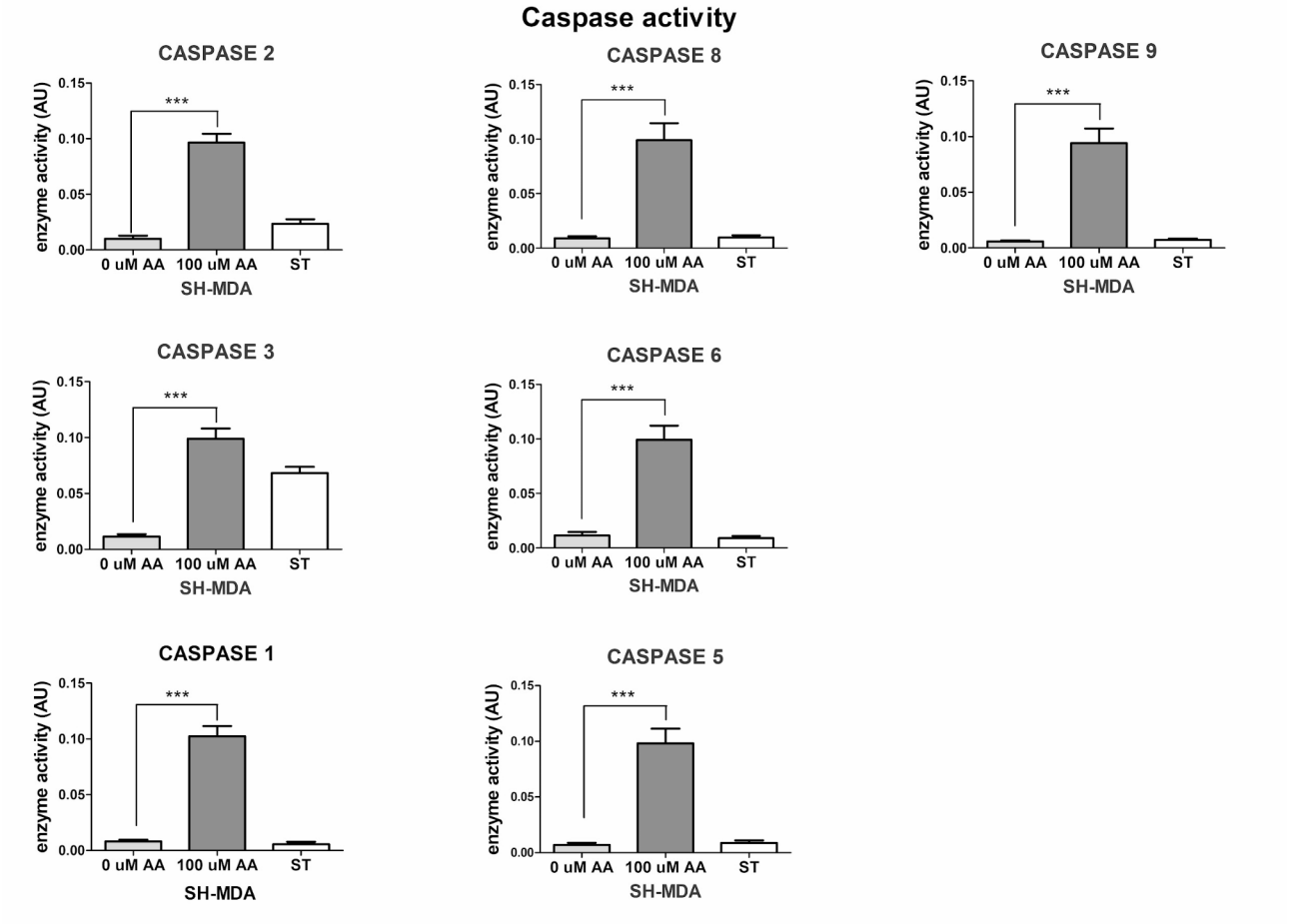

Fig. 3 AA increases the activity of several caspases in GPAT2-silenced cells. sh-MDA cells were treated with 100 μM AA for 48 h, and the activity of caspases 1, 2, 3, 5, 6, 8 and 9 was quantified using the colorimetric assay kit described in the Materials and Methods section. Staurosporine (ST) was used as a positive control for caspase-3 activation. The results are expressed as the mean ± SD of 3 independent experiments (***$P < 0.001$)

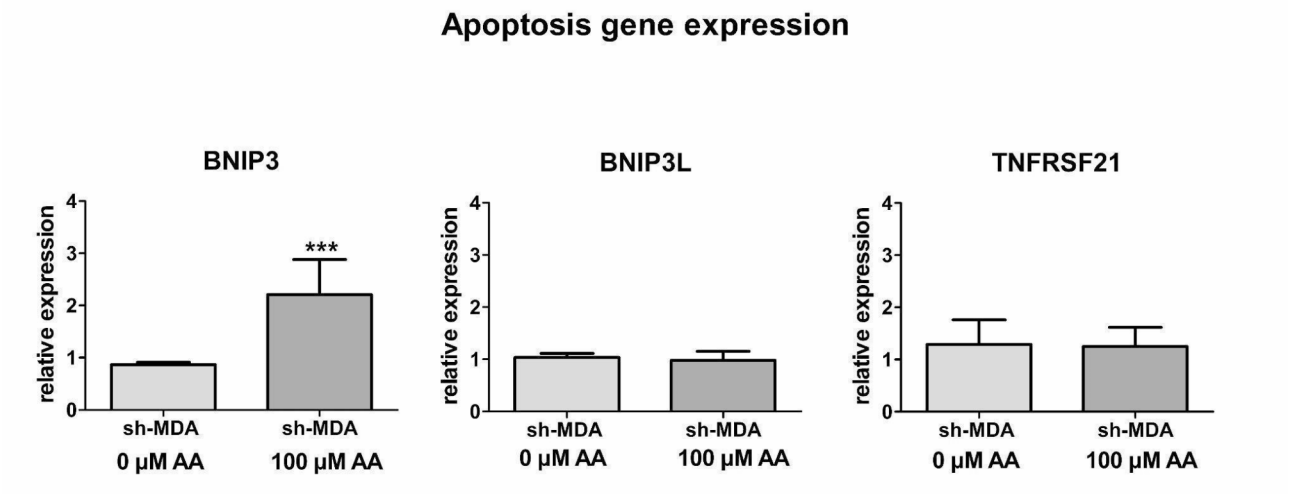

Fig. 4 Arachidonic acid increases the mRNA expression of the proapoptotic gene BNIP3. sh-MDA cells were treated with 100 μM AA for 48 h, after which BNIP3, BNIP3L and TNFRSF21 mRNA levels were analyzed via qRT–PCR. The values represent the means ± SDs of 3 independent experiments (***$P < 0.001$)

GPAT2 silencing changes AA metabolism-related gene expression

In breast cancer, AA-derived eicosanoids and their downstream pathways have been implicated in cell growth control and apoptotic pathways [13]. To understand the relationship between *GPAT2* expression and AA-induced apoptosis in sh-MDA cells the mRNA expression levels of genes involved in arachidonic acid utilization and eicosanoid biosynthesis, such as *AKR1C3* (encoding prostaglandin F synthase), *ALOX5* (encoding arachidonate 5-lipoxygenase), *EPHX2* (encoding epoxide hydrolase 2) and *PTGS2* (prostaglandin-Endoperoxide Synthase 2), were measured. We selected these genes taking into account our previous result from a microarray assay conducted on sh-MDA and scr-MDA cell lines, which showed differential expression of them [32]. Quantitative PCR results showed that sh-MDA cells significantly overexpressed the *ALOX5*, *EPHX2* and *AKR1C3* genes, while the PTGS2 gene expression decreased significantly, which could increase the vulnerability of sh-MDA cells to the action of these gene products (Fig. 5).

This observation suggested that GPAT2 expression is not only pivotal for preventing apoptosis triggered by free AA but also influences the expression of other AA-metabolizing genes.

Although mRNA levels do not always correlate with the amount of synthesized protein this measurement serves as an initial approach to understanding the potential effects of GPAT2 silencing on the expression of genes involved in eicosanoid biosynthesis.

On the other hand, AA exposure did not affect the expression levels of these genes in GPAT2 expressing cells, (Supplementary Material Fig. 2)

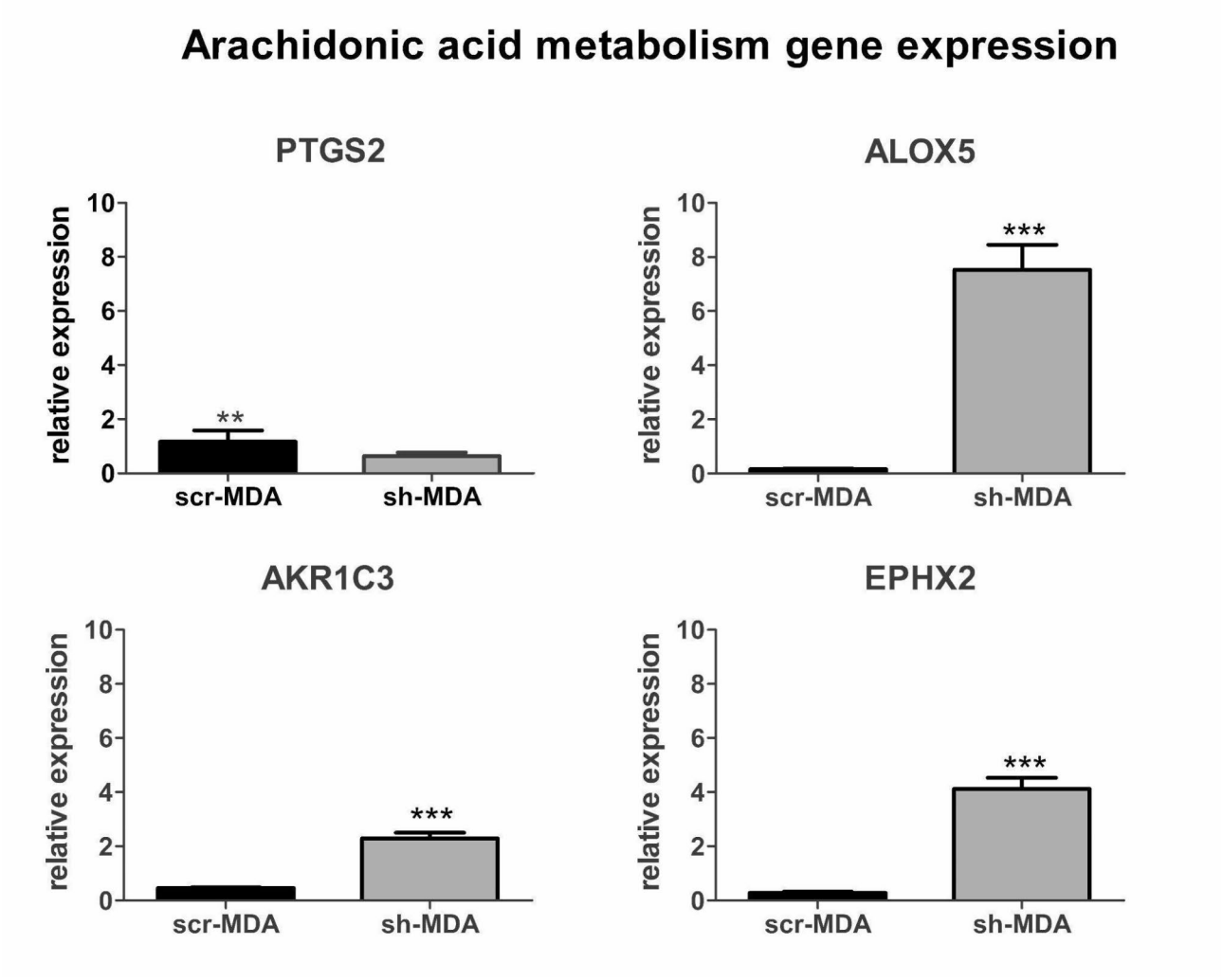

Fig. 5 GPAT2 silencing alters the mRNA expression of genes involved in arachidonic acid metabolism. Total RNA was extracted from scr-MDA and sh-MDA cells, subjected to cDNA synthesis, and amplified via qRT–PCR with primers for human PTGS2, ALOX5, AKR1C3 and EPHX2. The values represent the means ± SDs of 3 independent experiments (***$P < 0.001$, **$P < 0.01$)

Discussion

The primary finding of the present study was that GPAT2 enables breast cancer cells to evade AA-induced apoptosis.

Avoiding cell death by apoptosis is one of the hallmarks of cancer proposed by Douglas Hanahan in 2000 [10]. These original hallmarks of cancer have been reviewed and currently include several advantageous biological features acquired by cancer cells during the multistep development of human tumors [16, 17].

The relationship between AA and apoptosis is complex, and AA is reported to be either a proapoptotic or antiapoptotic signal, depending on the context and the specific signaling pathways involved. AA promotes apoptosis by activating certain enzymes, such as caspases, which are apoptotic effectors [3] or by inducing mitochondrial permeability transition [18]. Additionally, AA enables the production of reactive oxygen species, inducing apoptosis by damaging cellular components [19]. Conversely, AA also exerts antiapoptotic effects by triggering specific survival signaling pathways, such as the PI3K/Akt pathway [20, 21]. Additionally, AA can be converted into other bioactive lipids, such as prostaglandins and leukotrienes, which can have both pro- and antiapoptotic effects depending on their specific cellular targets and downstream signaling pathways [22].

However, there are certain types of tumors, such as breast and colon adenocarcinomas, in which it is well-known that exogenous AA induces apoptosis. These tumors overexpress the AA-utilizing enzymes ACSL4 and PTGS2. Because a high cellular level of unesterified AA is a general mechanism by which apoptosis is activated, the overexpression of these enzymes promotes carcinogenesis by lowering the level of this fatty acid [3, 13]. Moreover, *GPAT2* is overexpressed in breast (MDA-MB–231) and colon (HCT–116) cancer cell lines and, at the protein level, in human mammary adenocarcinomas of a higher histological grade [1]. Additionally, in a physiological context, *GPAT2* is highly expressed in pachytene spermatocytes, and silencing *Gpat2* in the mouse testis leads to reduced fertility and the overexpression of several genes involved in apoptotic pathways [23]. Because some of the overexpressed genes belonged to the *BLC2* family or membrane receptor family, the expression levels of *BNIP3*, *BNIP3L* and *TNFRSF21* were studied. As previously mentioned, AA treatment significantly increased *BNIP3* mRNA levels. BNIP3 (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3) belongs to the proapoptotic subfamily of proteins and is able to target mitochondria and cause apoptotic changes, such as a decrease in the membrane potential and the release of cytochrome c. The release of cytochrome c from mitochondria is one of the key events in apoptosis because cytochrome c can activate procaspase 9 and downstream caspases that amplify the death process [24-26].

BNIP3 plays a significant role in the regulation of cell death in many cancers, and its overexpression can be induced by various stimuli, including hypoxia and certain signaling pathways [26-28]. When *BNIP3* is overexpressed or induced by stress stimuli, it localizes to the mitochondrial membrane through its transmembrane domain, leading to an increase in ROS, the opening of the mitochondrial permeability transition pore, and the loss of mitochondrial membrane potential. *BNIP3* expression has also been associated with the response to chemotherapy in glioma cells [29] and leukemic cells [28]. Several studies have proposed that restoring *BNIP3* expression could increase the sensitivity of different tumor cells to chemical treatment [28]. However, regarding the particular effect of fatty acids on *BNIP3* expression, only a limited number of studies have been performed, and the results are dependent on the fatty acid studied and the cell type. For instance, in PC12 rat pheochromocytoma cells and in rat cortical cells, palmitic acid treatment increased *BNIP3* expression and apoptosis, while DHA alone did not alter *BNIP3* mRNA levels [30, 31].

In this study, AA treatment significantly increased BNIP3 expression (2.6-fold) in sh-MDA cells, potentially leading to an increase in caspase activity and explaining the proapoptotic effects of AA in GPAT2-silenced cells.

AA plays important roles in cell metabolism and is rapidly incorporated into the cell membrane phospholipids of mammalian cells or used as an eicosanoid precursor. Due to the potent biological actions of eicosanoids, cells maintain very low levels of AA by promoting their esterification into cell lipids. In a previous study of our group, sh-MDA cells exposed to 50 µM AA for 3 days were shown to overexpress AGPAT11 (the product of the LPCAT2 gene), another AA-metabolizing acyltransferase [32]. In this study, considering the results observed for cell viability, the expression levels of 4 genes encoding eicosanoid-producing enzymes were evaluated: PTGS2 (prostaglandin-Endoperoxide Synthase 2, or COX-2), ALOX5 (arachidonate 5-lipoxygenase), AKR1C3 (aldo-keto reductase family 1 member C3), and EPHX2 (epoxide hydrolase 2).

Eicosanoids comprise several hundred individual molecules, and their role in the regulation of apoptosis has been extensively studied in the context of tumorigenesis and cancer as well as in noncancerous tissues [33]. Eicosanoids can induce apoptosis through both extrinsic and intrinsic pathways [33], and variations in the levels and activity of enzymes that convert AA may lead to differences in the cellular concentrations of eicosanoids [34]. Accordingly, ACSL4 was shown to regulate the breast cancer cell phenotype through the products of ALOX5 and PTGS2 in the MDA-MB–231 and MCF–7 cell lines

[13]. In a colon cancer model, overexpression of both ACSL4 and PTGS2 led to a synergistic antiapoptotic effect [3], whereas hypoxia-related PTGS2 upregulation was associated with an increase in prostaglandin E2 levels [35]. In our *GPAT2*-silencing model, AA treatment significantly increased the activity of caspases in both the intrinsic and extrinsic pathways, as well as those associated with inflammatory processes. In addition, in sh-MDA cells, PTGS2 expression decreased 1.8-fold, ALOX5 expression increased 47-fold, AKR1C3 expression increased 4.9-fold and EPHX2 expression increased 14-fold. Although additional experiments are needed to determine the contribution of eicosanoids to the apoptotic process, changes in the expression levels of these AA-metabolizing enzymes may also explain the observed differences in cell survival in our sh-MDA cell model. (Additional information about the expression of these enzymes in breast cancer is included in the Supplementary Material).

The metabolic differences between tumor and non-tumor cells represent attractive targets for anticancer therapy. Our results, as well as those reported from other research groups, support the idea that alterations in the expression levels of enzymes involved in AA metabolic pathways impact cell survival [3, 13]. In this work, the effect of AA treatment on cell survival was studied in different GPAT2 expression contexts. It is proposed that, similar to other overexpressed enzymes involved in AA metabolism in tumors, GPAT2 is necessary to evade apoptosis induced by free AA. In cells expressing GPAT2 (scr-MDA), most of the AA is esterified into triacylglycerols, decreasing the level of this pro-apoptotic agent. While when GPAT2 expression is silenced, MDA-MB-231 cells compensate for its absence by overexpressing other enzymes capable of utilizing AA as a substrate, such as AGPAT11, ALOX5, AKR1C3, and EPHX2. This leads to metabolic reprogramming that allows cells to survive when AA levels are lower (routine culture medium). However, under conditions of high concentrations of AA, these enzymes cannot fully compensate for the absence of GPAT2; and as a result, the eicosanoids produced, the overexpression of BNIP3, or both leads to apoptosis in sh-MDA cells (Fig. 6).

Study strengths and limitations

The greatest strength of this study is the assessment of the role of GPAT2 in AA metabolism in triple negative breast cancer. The use of the well-characterized MDA-MB-231 breast cancer cell line, which enabled precise manipulation of GPAT2 expression, allowed a detailed examination of its role in AA-induced apoptosis. The employment of diverse and complementary assays,

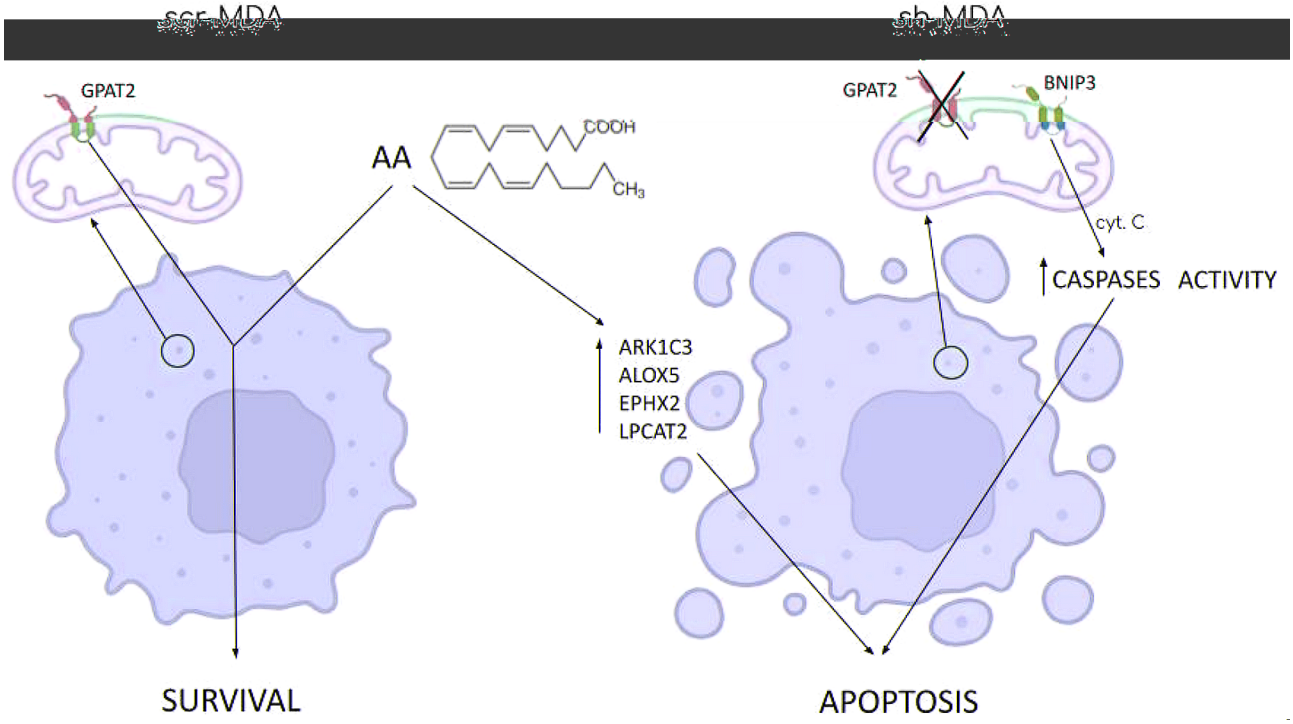

Fig. 6 Schematic model of arachidonic acid-induced apoptosis in GPAT2-silenced cells. The cellular level of unesterified arachidonic acid is a general mechanism by which apoptosis is regulated, and GPAT2 promotes carcinogenesis by decreasing this level. In scr-MDA cells, GPAT2 esterifies arachidonoyl-CoA to glycerol–3-phosphate, allowing these cells to survive AA treatment. Instead, sh-MDA cells overexpress eicosanoid-producing enzymes, and AA increases BNIP3 expression, which leads to apoptosis in GPAT2-silenced cells. (cyt. C: cytochrome C, AA: arachidonic acid)

such as MTT, TUNEL, and caspase activity measurements, provided robust and comprehensive data. Furthermore, the exploration of molecular mechanisms through qRT-PCR analysis enriched the understanding of GPAT2's function in apoptotic pathways. In breast cancer research, it is common to use more than one cell line model, and another frequently used model is the MCF7 cell line. Although MCF7 cells do not express GPAT2 [1], they also lack caspase-3 [36], one of the key caspases involved in AA-induced apoptosis [3, 5, 18]. For this reason, we did not consider this cell line appropriate for our studies.

The study also has some limitations. The in vitro nature of the experiments may not fully replicate the complexity of the tumor microenvironment in vivo, which could affect the cells' behavior and treatment responses. Future studies involving animal models and a protein expression rescue assay are necessary to validate and extend these findings.

Conclusion

The present study indicates that breast cancer cells lacking GPAT2 expression are more sensitive to AA-induced apoptosis, suggesting that AA could be considered as part of the treatment regimen for GPAT2-negative breast cancers. This finding is particularly relevant given that approximately 63% of breast adenocarcinomas do not express GPAT2, and supports our previous results that implicate GPAT2 in the regulation of apoptotic pathways and the development of a tumorigenic phenotype. Utilizing AA in combination with other treatments might enhance the effectiveness of therapies aimed at inducing apoptotic cell death in these specific breast cancer subtypes. Future research should focus on validating this approach in vivo and exploring its clinical implications to improve treatment outcomes for patients with GPAT2-negative breast cancers.

Abbreviations

AA	Arachidonic Acid
GPAT	Glycerol-3-Phosphate Acyltransferase
qRT–PCR	Quantitative Real-Time PCR
scr-MDA	MDA-MB-231 cells that express GPAT2
sh-MDA	MDA-MB-231 cells with reduced GPAT2 expression

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12944-024-02344-1.

Supplementary Material 1

Acknowledgements

We thank Marianela Santana and Leticia Aguirre for their technical assistance.

Author contributions

Fiorella J. Ferremi: Formal analysis, Investigation. Veronica V. Moscoso: Formal analysis, Investigation. Mauro A. Montanaro: Investigation, Writing – review & editing. Maria R. Gonzalez- Baro: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. Elizabeth R. Cattaneo: Conceptualization, Formal analysis, Supervision, Writing – original draft, Writing – review & editing.

Funding

This work was supported by: Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) PIP N°0470. UNLP 11 M/241.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

Received: 2 July 2024 / Accepted: 22 October 2024
Published online: 27 November 2024

References

1. Pellon-Maison M, et al. Glycerol–3-phosphate acyltranferase–2 behaves as a cancer testis gene and promotes growth and tumorigenicity of the breast cancer MDA-MB–231 cell line. PLoS ONE. 2014;9(6):1–11. https://doi.org/10.1371/journal.pone.0100896.
2. Cattaneo ER, Pellon-Maison M, Rabassa ME, Lacunza E, Coleman RA, Gonzalez-Baro MR. Glycerol–3-phosphate acyltransferase–2 is expressed in spermatic germ cells and incorporates arachidonic acid into triacylglycerols. PLoS ONE. 2012;7(8):1–11. https://doi.org/10.1371/journal.pone.0042986.
3. Cao Y, Pearman AT, Zimmerman GA, McIntyre TM, Prescott SM. Intracellular unesterified arachidonic acid signals apoptosis, Proc. Natl. Acad. Sci. U. S. A., vol. 97, no. 21, pp. 11280–11285, 2000, https://doi.org/10.1073/pnas.200367597
4. Li J, et al. Berberine induces apoptosis by suppressing the arachidonic acid metabolic pathway in hepatocellular carcinoma. Mol Med Rep. 2015;12(3):4572–7. https://doi.org/10.3892/mmr.2015.3926.
5. Monjazeb AM, High KP, Connoy A, Hart LS, Koumenis C, Chilton FH. Arachidonic acid-induced gene expression in colon cancer cells. Carcinogenesis. 2006;27(10):1950–60. https://doi.org/10.1093/carcin/bgl023.
6. Monjazeb AM, High KP, Koumenis C, Chilton FH. Inhibitors of arachidonic acid metabolism act synergistically to signal apoptosis in neoplastic cells. Prostaglandins Leukot Essent Fat Acids. 2005;73(6):463–74. https://doi.org/10.1016/j.plefa.2005.07.009.
7. Greenwald P. Cancer prevention clinical trials. J Clin Oncol, 20, no. 18 SUPPL., 2002.
8. Yarla NS, Targeting arachidonic acid pathway by natural products for cancer prevention and therapy, Semin. Cancer Biol., vol., 40_41 et al. no. 2016, pp. 48–81, 2016, https://doi.org/10.1016/j.semcancer.2016.02.001
9. Yin H, et al. Role of mitochondria in programmed cell death mediated by arachidonic acid-derived eicosanoids. Mitochondrion. 2013;13(3):209–24. https://doi.org/10.1016/j.mito.2012.10.003.
10. Douglas Hanahan, Weinberg RA. The Hallmarks of Cancer. Cell. 2000;100:57–70. https://doi.org/10.1107/S2059798322003928.
11. Orafaie A, Mousavian M, Orafai H, Sadeghian H. An overview of lipoxygenase inhibitors with approach of in vivo studies. Prostaglandins Other Lipid Mediat. 2020;148:106411. https://doi.org/10.1016/j.prostaglandins.2020.106411.
12. Dattilo MA, et al. Regulatory mechanisms leading to differential Acyl-CoA synthetase 4 expression in breast cancer cells. Sci Rep. 2019;9(1):1–13. https://doi.org/10.1038/s41598-019-46776-7.
13. Maloberti PM, et al. Functional interaction between acyl-coa synthetase 4, lipooxygenases and cyclooxygenase–2 in the aggressive phenotype of breast cancer cells. PLoS ONE. 2010;5(11). https://doi.org/10.1371/journal.pone.0015540.
14. Mosmann T. Rapid Colorimetric Assay for Cellular Growth and Survival: application to proliferation and cytotoxicity assays. J Lmmunological Methods. 1983;65(83):55–63. https://doi.org/10.1016/0022-1759(83)90303-4.

15. Leaver HA, et al. Antitumour and pro-apoptotic actions of highly unsaturated fatty acids in glioma. Prostaglandins Leukot Essent Fat Acids. 2002;66(1):19–29. https://doi.org/10.1054/plef.2001.0336.
16. Hanahan D. Hallmarks of Cancer: New dimensions. Cancer Discov. 2022;12(1):31–46. 10.1158/2159–8290.CD–21–1059.
17. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74. https://doi.org/10.1016/j.cell.2011.02.013.
18. Scorrano L, Penzo D, Petronilli V, Pagano F, Bernardi P. Arachidonic acid causes cell death through the mitochondrial permeability transition. Implications for tumor necrosis factor-α apoptotic signaling. J Biol Chem. 2001;276(15):12035–40. https://doi.org/10.1074/jbc.M010603200.
19. Chen KC, Sen Chang L. Arachidonic acid-induced apoptosis of human neuroblastoma SK-N-SH cells is mediated through mitochondrial alteration elicited by ROS and Ca2+-evoked activation of p38α MAPK and JNK1. Toxicology. 2009;262(3):199–206. https://doi.org/10.1016/j.tox.2009.06.009.
20. Chénais B, Blanckaert V. The Janus face of lipids in human breast Cancer: how polyunsaturated fatty acids affect Tumor Cell Hallmarks. Int J Breast Cancer. 2012;2012:1–8. https://doi.org/10.1155/2012/712536.
21. Li Y, et al. Isoliquiritigenin induces growth inhibition and apoptosis through downregulating arachidonic acid metabolic network and the deactivation of PI3K/Akt in human breast cancer. Toxicol Appl Pharmacol. 2013;272(1):37–48. https://doi.org/10.1016/j.taap.2013.05.031.
22. Montecillo-Aguado M, Tirado-Rodriguez B, Huerta-Yepez S. The involvement of Polyunsaturated fatty acids in apoptosis mechanisms and their implications in Cancer. Int J Mol Sci. 2023;24(14). https://doi.org/10.3390/ijms241411691.
23. Garcia-Fabiani MB, et al. Glycerol–3-phosphate acyltransferase 2 is essential for normal spermatogenesis. Biochem J. 2017;474:3093–107. https://doi.org/10.1042/BCJ20161018.
24. Madhu V, et al. The mitophagy receptor BNIP3 is critical for the regulation of metabolic homeostasis and mitochondrial function in the nucleus pulposus cells of the intervertebral disc. Autophagy. 2023;19(6):1821–43. https://doi.org/10.1080/15548627.2022.2162245.
25. Petronilli V, Penzo D, Scorrano L, Bernardi P, Di Lisa F. The mitochondrial permeability transition, release of cytochrome c and cell death. Correlation with the duration of pore openings in situ. J Biol Chem. 2001;276(15):12030–4. https://doi.org/10.1074/jbc.M010604200.
26. Shao Y, et al. Expression and epigenetic regulatory mechanism of BNIP3 in clear cell renal cell carcinoma. Int J Oncol. 2019;54(1):348–60. https://doi.org/10.3892/ijo.2018.4603.
27. Burton TR, Gibson SB. The role of Bcl–2 family member BNIP3 in cell death and disease: NIPping at the heels of cell death. Cell Death Differ. 2009;16(4):515–23. https://doi.org/10.1038/cdd.2008.185.
28. Cavaliere V, Lombardo T, Costantino SN, Kornblihtt L, Alvarez EM, Blanco GA. Synergism of arsenic trioxide and MG132 in Raji cells attained by targeting BNIP3, autophagy, and mitochondria with low doses of valproic acid and vincristine. Eur J Cancer. 2014;50(18):3243–61. https://doi.org/10.1016/j.ejca.2014.09.012.
29. Kanzawa T, Zhang L, Xiao L, Germano IM, Kondo Y, Kondo S. Arsenic trioxide induces autophagic cell death in malignant glioma cells by upregulation of mitochondrial cell death protein BNIP3. Oncogene. 2005;24(6):980–91. https://doi.org/10.1038/sj.onc.1208095.
30. Frankis MDL, Almaguel1 G, Liu J-W 1. Fabio J. Pacheco1, 2, Carlos A. Casiano1, Activation and Reversal of Lipotoxicity in PC12 and Rat Cortical Cells Following Exposure to Palmitic Acid, J Neurosci Res, vol. 87, no. 5, pp. 1207–1218, 2009, https://doi.org/10.1002/jnr.21918.Activation
31. Montero ML, Liu JW, Orozco J, Casiano CA, De Leon M. Docosahexaenoic acid protection against palmitic acid-induced lipotoxicity in NGF-differentiated PC12 cells involves enhancement of autophagy and inhibition of apoptosis and necroptosis. J Neurochem. 2020;155(5):559–76. https://doi.org/10.1111/jnc.15038.
32. Cattaneo ER, Prieto ED, Garcia-Fabiani MB, Montanaro MA, Guillou H, Gonzalez-Baro MR. Glycerol–3-phosphate acyltransferase 2 expression modulates cell roughness and membrane permeability: an atomic force microscopy study. PLoS ONE. 2017;12(12):1–18. https://doi.org/10.1371/journal.pone.0189031.
33. Borin TF, Angara K, Rashid MH, Achyut BR, Arbab AS. Arachidonic acid metabolite as a novel therapeutic target in breast cancer metastasis. Int J Mol Sci. 2017;18(12):2661.
34. Villegas-Comonfort S, Castillo-Sanchez R, Serna-Marquez N, Cortes-Reynosa P, Salazar EP. Arachidonic acid promotes migration and invasion through a PI3K/Akt-dependent pathway in MDA-MB–231 breast cancer cells. Prostaglandins Leukot Essent Fat Acids. 2014;90(5):169–77. https://doi.org/10.1016/j.plefa.2014.01.007.
35. Kaidi A, Qualtrough D, Williams AC, Paraskeva C. Direct transcriptional up-regulation of cyclooxygenase–2 by hypoxia-inducible factor (HIF)–1 promotes colorectal tumor cell survival and enhances HIF–1 transcriptional activity during hypoxia. Cancer Res. 2006;66(13):6683–91. https://doi.org/10.1158/0008-5472.CAN-06-0425.
36. Jänicke RU. MCF-7 breast carcinoma cells do not express caspase-3. Breast Cancer Res Treat. 2009;117(1):219–21. https://doi.org/10.1007/s10549-008-0217-9. Epub 2008 Oct 14. PMID: 18853248.

Publisher's note