**Name of Journal:** *World Journal of Clinical Oncology*

**Manuscript NO:** 74097

**Manuscript Type:** ORIGINAL ARTICLE

***Basic Study***

**Nicotinic receptors modulate antitumor therapy response in triple negative breast cancer cells**

Español A *et al*. Nicotine as a modulator of chemotherapy

Alejandro Español, Yamila Sanchez, Agustina Salem, Jaqueline Obregon, Maria Elena Sales

**Alejandro Español, Yamila Sanchez, Agustina Salem, Jaqueline Obregon, Maria Elena Sales,** Laboratory of Immunopharmacology and Tumor Biology, CEFYBO CONICET University of Buenos Aires, Buenos Aires C1121ABG, Argentina

**Author contributions:** Español A performed cell assays, supervised the work, analyzed and interpreted the data, and wrote the manuscript; Sanchez Y and Salem A performed cell assays and contributed to the writing of the manuscript; Obregon J carried out lab work as part of her grade thesis, and helped to analyze and interpret the data; Sales ME supervised the work and edited the manuscript draft; all authors read and approved the final manuscript.

**Supported by** University of Buenos Aires (UBA) UBACYT 2018-2022, No. 20020170100227; National Research Council (CONICET) PIP 2015-2017, No. 2015-0239; and National Agency for Scientific and Technological Promotion (ANPCyT) PICT 2015-2017, No. 2015-2396.

**Corresponding author: Alejandro Español, PhD, Research Scientist,** Laboratory of Immunopharmacology and Tumor Biology, CEFYBO CONICET University of Buenos Aires, Paraguay 2155 16th Floor, Buenos Aires C1121ABG, Argentina. aespan\_1999@yahoo.com

**Received:** December 27, 2021

**Revised:** February 24, 2022

**Accepted:** April 26, 2022

**Published online:**

**Abstract**

BACKGROUND

Triple negative breast cancer is more aggressive than other breast cancer subtypes and constitutes a public health problem worldwide since it has high morbidity and mortality due to the lack of defined therapeutic targets. Resistance to chemotherapy complicates the course of patients’ treatment. Several authors have highlighted the participation of nicotinic acetylcholine receptors (nAChR) in the modulation of conventional chemotherapy treatment in cancers of the airways. However, in breast cancer, less is known about the effect of nAChR activation by nicotine on chemotherapy treatment in smoking patients.

AIM

To investigate the effect of nicotine on paclitaxel treatment and the signaling pathways involved in human breast MDA-MB-231 tumor cells.

METHODS

Cells were treated with paclitaxel alone or in combination with nicotine, administered for one or three 48-h cycles. The effect of the addition of nicotine (at a concentration similar to that found in passive smokers’ blood) on the treatment with paclitaxel (at a therapeutic concentration) was determined using the 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The signaling mediators involved in this effect were determined using selective inhibitors. We also investigated nAChR expression, and ATP “binding cassette” G2 drug transporter (ABCG2) expression and its modulation by the different treatments with Western blot. The effect of the treatments on apoptosis induction was determined by flow cytometry using annexin-V and 7AAD markers.

RESULTS

Our results confirmed that treatment with paclitaxel reduced MDA-MB-231 cell viability in a concentration-dependent manner and that the presence of nicotine reversed the cytotoxic effect induced by paclitaxel by involving the expression of functional α7 and α9 nAChRs in these cells. The action of nicotine on paclitaxel treatment was linked to modulation of the protein kinase C, mitogen-activated protein kinase, extracellular signal-regulated kinase, and NF-κB signaling pathways, and to an up-regulation of ABCG2 protein expression. We also detected that nicotine significantly reduced the increase in cell apoptosis induced by paclitaxel treatment. Moreover, the presence of nicotine reduced the efficacy of paclitaxel treatment administered in three cycles to MDA-MB-231 tumor cells.

CONCLUSION

Our findings point to nAChRs as responsible for the decrease in the chemotherapeutic effect of paclitaxel in triple negative tumors. Thus, nAChRs should be considered as targets in smoking patients.

**Key Words:** Breast cancer; Paclitaxel; Nicotinic acetylcholine receptors; Drug therapy; Signal transduction; Drug transporter

Español A, Sanchez Y, Salem A, Obregon J, Sales ME. Nicotinic receptors modulate antitumor therapy response in triple negative breast cancer cells. *World J Clin Oncol* 2022; In press

**Core Tip:** Smokers with lung tumors are more likely to generate resistance to chemotherapy than non-smokers. However, little is known about the effect of nicotinic activation during the treatment of breast cancer, a cancer which arises close to the lung. In triple negative human breast cells, nicotine reduces the chemotherapeutic effect of paclitaxel through the participation of several kinases, as well as by modulating ATP “binding cassette” G2 drug transporter expression and inducing resistance to treatment. These results indicate that nicotinic acetylcholine receptors are a new possible target in antitumor therapy for this subtype of breast cancer.

**INTRODUCTION**

Cigarette smoke contains many harmful components for human health[1]. Among them, nicotine (NIC), which has addictive properties[2], exerts its effects by activating nicotinic acetylcholine receptors (nAChRs)[3]. These receptors belong to the Cys-loop family of pentameric ionic channels activated by ligands[4]. nAChRs can be made up of 17 different subunits, whose assembly creates several homopentameric and heteropentameric channel subtypes in the cell membrane[5].

Originally, nAChRs were found in the nervous system. However, their expression has been described in several organs such as the lungs[6,7], kidney[8,9], intestine[10,11] and breast, where mainly the α7 and α9 subunits are expressed[12,13]. The activation of nAChRs can induce an increase in the levels of intracellular calcium[14], which has been related to tumorigenesis in the lungs[15], liver[16], pancreas[17] and brain[18]. This increase in intracellular calcium can in turn activate kinase signaling pathways[19,20], which regulate different parameters of tumor biology such as proliferation, migration and invasion[21-23].

Previous evidence has demonstrated that the activation of these receptors can decrease the effectiveness of different antitumor agents in various tumor types such as those from the oral and nasal cavity[14,24], pancreas[25], head and neck[26] and lungs[27-29]. In the lungs, many authors have described that NIC may exert its modulatory effect on the actions of chemotherapeutic agents through the activation of signaling pathways involving protein kinases[30-33]. However, little is known about the effect of nicotinic activation during the treatment of breast cancer, a cancer that arises close to the lungs.

Breast cancer is characterized by a high incidence that causes a high number of deaths in women worldwide[34]. According to their genetic profile, breast tumors are classified in different subtypes, a fact that allows doctors to choose the most effective antitumor therapy. Tumors can be categorized according to the expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki-67 protein by immunohistochemistry. This allows defining four subtypes of breast tumors: luminal A, luminal B, HER2+ and normal-like or triple negative (TN)[35,36]. TN tumors lack the expression of ER, PR and HER2, but present high expression of Ki-67 protein and are usually very invasive and aggressive[37]. Since these tumors do not exhibit a defined therapeutic target, there is no specific treatment, and they respond poorly to taxanes like paclitaxel (PX), usually administered as conventional chemotherapy to other breast cancer patients.

PX is an effective drug used not only in the treatment of breast cancer, but also in non-small cell lung cancer, prostate cancer, and head and neck cancer[38]. It is a cytostatic compound that causes hyper-stabilization of polymerized microtubules, inhibiting the mitotic spindle and arresting cells in G2/M phases[39]. The persistence of cell arrest eventually produces cell death by apoptosis[40]. Thus, a low level of apoptosis could be an important factor in the development of resistance to treatment.

In this study, we evaluated the ability of NIC to interfere in the treatment of human TN breast cancer MDA-MB-231 cells with PX and the signaling pathway involved in this action.

**MATERIALS AND METHODS**

***Cell culture***

The human breast adenocarcinoma cell lines MDA-MB-231 (TN, CRM-HTB-26), MDA-MB-468 (TN, HTB-132) and MCF-7 (luminal A, HTB-22) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, United States) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen Inc., Carlsbad, CA, United States) supplemented with 100 mL/L heat inactivated fetal bovine serum (FBS) (Internegocios SA, Mercedes, Buenos Aires, Argentina), L-glutamine (0.3 mg/L) and gentamicin (80 mg/L). The cultures were maintained at 37 °C in humidified air with 50 mL/L CO2 and the medium was replaced three times a week. Cells were detached with 250 mg/L trypsin in Ca2+- and Mg2+-free PBS containing 20 mg/L EDTA. Cell viability was determined by the Trypan blue exclusion test and the absence of mycoplasma was observed by Hoechst staining[41].

***Cell viability assay***

Cell viability after treatment was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining method (Life Technologies, Eugene, OR, United States). A suspension containing 4 × 103 cells/well was added to each well of a 96-well plate in culture medium supplemented with 50 mL/L FBS and cells were then left to attach overnight. When cells reached 60%-70% of confluence, they were deprived of FBS for 24 h to induce the synchronization of cultures. Then, cells were treated with PX (Bristol-Myers Squibb, Buenos Aires, Argentina) and/or NIC (which is a non-selective nAChR agonist) in medium supplemented with 20 mL/L FBS, for 48 h in triplicate. To inhibit the action of the nicotinic agonist, cells were previously treated with the antagonists mecamylamine (MM), methyllycaconitine (MLA) or luteolin (Lut) at 10-6 mol/L; these three are respectively nAChR non-selective, α7 nAChR selective and α9 nAChR selective antagonists. To determine the participation of kinases in the effects of PX, cells were previously treated with inhibitors of: protein kinase C (PKC) [staurosporine (Stau), 10-8 mol/L], mitogen-activated protein kinase kinase (MEK) [PD098059 (PD), 10-5 mol/L], Ras [S-trans, trans-farnesylthiosalicylic acid (FTS), 10-6 mol/L], extracellular signal-regulated kinases (ERK1/2) (U126, 10-5 mol/L), p38 mitogen-activated protein kinases (p38MAPK) [SB203580 (SB), 10-5 mol/L] or the mediator of the activation of the NF-κB pathway IκB kinase (IKKβ) [IMD354 (IMD), 5 × 10-8 mol/L].

To determine the modulation of the sensitivity of MDA-MB-231 cells to chemotherapy, cells were treated with PX in the absence or presence of NIC for three 48-h cycles with 24 h intercycles without treatment. Then, surviving cells were treated with a new cycle of PX for 48 h. After treatment, the medium was removed and 100 μL of MTT solution (500 mg/L medium free of phenol red and FBS) was added. Plates were incubated for 4 h at 37°C and the production of formazan was measured by analyzing the absorbance at 540 nm with an ELISA reader (BioTek, Winooski, VT, United States). Values are indicated as mean ± SD and expressed as the percentage of cell viability in comparison to cells without treatment considered 100%.

A diagram of the administration schedule for the determination of cell viability or cell sensitivity to chemotherapy is shown in Figure 1.

***Calculation of the effective concentration 50***

Dose–response data were transformed, changed to percentage and fitted to a sigmoidal curve, following a maximal effective concentration (Emax) model with at least six data points, using the GraphPad Prism 6 software. This allowed calculating the effective concentration 50 (EC50) and Emax values. Only data with a coefficient of variation lower than 20% were considered for the EC50 values.

***Detection of nicotinic receptors by Western blot***

MDA-MB-231 cell proteins were extracted by washing them with a buffer containing 6 g/L Tris-HCl, 3 g/L NaCl, 210 mg/L NaF, 480 mg/L MgCl2, 300 mg/L EDTA, 380 mg/L EGTA, 870 mg/L phenylmethanesulfonyl fluoride, 10 mL/L Triton X-100 and 10 mg/L trypsin inhibitor, aprotinin and leupeptin, at pH 7.4. Samples were incubated on ice for 1 h and centrifuged at 800 G for 20 min at 4°C, afterwards the supernatants were collected and saved at -80°C. Protein concentrations were determined by the Bradford assay[42].

Samples (80 μg protein per lane) were separated by 10% SDS-PAGE minigel electrophoresis and then transferred to nitrocellulose membranes. They were then blocked with 50 g/L skim milk and incubated overnight with rat anti-human-α7 nAChRs monoclonal antibody or mouse anti-human-α9 nAChRs monoclonal antibody (Santa Cruz Biotechnology Inc., Dallas, TX, United States), both diluted 1:200. Then, strips were incubated with anti-rat or anti-mouse IgG coupled to horseradish peroxidase diluted 1:10000 in buffer containing 2.4 g/L Tris-HCl buffer, 9 g/L NaCl and 500 mg/L Tween 20 (TBS-T) at 37°C for 1 h. α7 nAChR and α9 nAChR bands were detected by chemiluminescence and quantified by densitometric analysis using Image J software (NIH). The results are expressed as optical density (O.D.) units relative to the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Santa Cruz Biotechnology Inc., Dallas, TX, United States), which was used as the loading control[43].

***Trypan blue exclusion assay***

Cell viability was also determined by the trypan blue dye exclusion test. Briefly, cells were treated with the corresponding drugs for 48 h in 48-well plates at a density of 104 cells/well. Cells were collected and centrifuged at 900 r/min for 10 min. Then, pellets were resuspended in DMEM and the trypan blue solution was added in a 1:1 ratio. The number of viable cells, identified as non-stained cells, was counted using a hemocytometer under an inverted microscope at 10X magnification and the percentage of these cells with respect to the total cell number was calculated.

***Apoptosis determination by flow cytometry***

For apoptosis determination, MDA-MB-231 cells were grown in six-well plates and treated for 48 h with PX, NIC or their combination, in the presence or absence of selective and non-selective nicotinic antagonists and kinase inhibitors. Then, cells were harvested and resuspended in binding buffer, and 2 μL of AnnexinV-FITC was added to each sample. Cells were then incubated for 15 min at room temperature in the dark. After that, 2 μL of 7AAD was added and the samples were immediately analyzed by the BD Accuri C6 Plus Flow Cytometer. Data were analyzed by the BD Accuri C6 Plus software.

***ATP binding cassette transporter G2 detection by Western blot***

For the detection of ATP “binding cassette” G2 drug transporter (ABCG2), cells (2 × 106) were treated for 48 h with the different drugs and samples were prepared as indicated to detect nicotinic receptors. Then, samples (80 μg protein per lane) were separated by 12% SDS-PAGE minigel electrophoresis, transferred to nitrocellulose membranes, blocked with 50 g/L skim milk and incubated overnight with a rabbit anti-human ABCG2 polyclonal antibody (Santa Cruz Biotechnology Inc., Dallas, TX, United States) diluted 1:200. Then, strips were incubated with horseradish peroxidase-linked anti-rabbit IgG, diluted 1:10000 in TBS-T at 37°C for 1 h. Bands were detected by chemiluminescence and then quantified by densitometric analysis using the Image J program (NIH) and expressed as O.D. units in comparison to the expression of GAPDH, which was used as the loading control[44].

***Statistical analysis***

Results are expressed as mean ± SD and statistical analysis was performed using the GraphPad Prism6 software. To determine differences between mean values, one-way ANOVA was performed with Tukey´s post-hoc analysis. *P* < 0.05 was accepted as statistically significant. The data and statistical analysis complied with the recommendations on experimental design and analysis in pharmacology[45].

**RESULTS**

***Effect of NIC on MDA-MB-231 cell viability***

First, we analyzed the effect of NIC administered for 48 h to MDA-MB-231 cells in culture. The addition of different concentrations of NIC increased cell viability from 10-10 mol/L, with an Emax of 189.3% ± 5.2% (Figure 2A). This effect was reduced by the pre-treatment of cells with different nicotinic antagonists: MM (non-selective) (Emax: 118.4% ± 3.36%), MLA (α7 nAChR selective) (Emax: 143.6% ± 1.38%), or Lut (α9 nAChR selective) (Emax: 135.6% ± 5.69%), all of them added at 10-6 mol/L (all *P* < 0.001 *vs* NIC treatment) (Figure 2A). The nicotinic antagonists alone did not modify cell viability (data not shown). In addition, expression of α7 and α9 nAChRs in MDA-MB-231 cells was detected by Western blot assay (Figure 2B).

***Paclitaxel treatment of MDA-MB-231 cells in the presence of NIC***

In the next set of experiments, we analyzed the action of PX on tumor cells in the presence of NIC. First, we confirmed that PX reduced MDA-MB-231 cell viability in a concentration-dependent manner and that the PX effect was significant at concentrations equal to or higher than 10-8 mol/L (EC50: 1.0 × 10-7 mol/L) (Figure 3). On the other hand, the presence of the first effective concentration of NIC (10-10 mol/L) shifted the concentration-response curve to the right, increasing the EC50 value by more than one order (EC50: 1.2 × 10-6 mol/L) (Figure 3).

After three cycles of PX (48 h each) in the presence of NIC, followed by 24 h without drugs, surviving cells were less sensitive to a subsequent PX cycle in comparison to the same treatment in the absence of NIC. The latter was evidenced by an increase in the EC50 value obtained from the concentration-response-curve (PX+NIC EC50: 3.6 × 10-6 mol/L; PX EC50: 4.8 × 10-7 mol/L) (Figure 4).

It has been documented that 10-10 mol/L can be considered a concentration of NIC similar to that present in the bloodstream of passive smoking patients[46]. To analyze the ability of NIC to interfere with the action of therapeutic concentrations of PX, we treated MDA-MB-231 cells with a combination of 10-10 mol/L NIC and 10-7 mol/L PX for 48 h. We determined that NIC reduced PX effectiveness by increasing tumor cell viability to 110.8% ± 1.9% in comparison to PX treatment in the absence of NIC (51.1% ± 3.9%, *P* < 0.01). This effect of NIC on PX action was partially reduced by the pre-treatment of cells with nicotinic antagonists (MM, MLA or Lut) added at 10-6 mol/L (*P* < 0.001 *vs* PX+NIC) (Figure 5A) in a manner similar to that of NIC treatment alone (NIC: 138.16 ± 6.08; NIC+MM: 116.52 ± 0.12; NIC+Lut: 126.16 ± 5.96; NIC+MLA: 119.69 ± 4.26; *P* < 0.001; *P* < 0.05 and *P* < 0.001 *vs* NIC, respectively). We confirmed that the PX effect was independent of nAChR activation since pre-treatment with nicotinic antagonists did not modify the effect of PX treatment alone (PX+MM: 52.12 ± 6.65; PX+Lut: 49.97 ± 5.88; PX+MLA: 54.6 ± 4.01). To confirm the action of drugs on cell viability, we next analyzed the ratio of living cells by the Trypan blue exclusion test after the different treatments. The results plotted in Figure 5B (control: 100 ± 3.3; NIC: 129.3 ± 1.1, *P* < 0.001; PX: 64.1 ± 4.3, *P* < 0.001; PX+NIC: 92.3 ± 2.5, P=ns; PX+NIC+MM: 56.3 ± 3.4, *P* < 0.001; PX+NIC+MLA: 66.7 ± 5.4, *P* < 0.001; PX+NIC+Lut: 52.3 ± 6.0, *P* < 0.001; all being % or significance with respect to the control) show values similar to those obtained by the MTT assay (Figure 5A).

To confirm that nicotinic agonists can modulate the action of PX in reducing viability in other breast cancer cells, we tested the effect of PX (10-7 mol/L) in the absence or presence of NIC (10-10 mol/L) on MDA-MB-468 and MCF-7 cell viability. We determined that the presence of NIC reduced the effect of PX in MDA-MB-468 and MCF-7 cells, and that these effects were prevented by pre-incubating cells with 10-6 mol/L of MM (Table 1).

***Signal transduction pathways and mechanism involved in the effect of paclitaxel on NIC-treated tumor cells***

Previous reports have indicated that the activation of protein kinases is essential to modulate cell viability by triggering pro/anti-apoptotic gene transcription[47,48]. Additionally, the induction of the expression of these proteins could implicate activation of the NF-κB pathway. In the present study, we observed that the effect of PX on MDA-MB-231 cells is mediated, at least in part, by the Ras, p38MAPK (*P* < 0.01 *vs* PX) and NF-κB pathways (*P* < 0.05 *vs* PX) since the addition of their specific inhibitors FTS, SB or IMD respectively modified PX action (Figure 6A). On the other hand, the presence of NIC during PX treatment not only involved the previously mentioned molecules, but also the PKC (*P* < 0.05 *vs* PX), MEK (*P* < 0.01 *vs* PX) and ERK1/2 (*P* < 0.01 *vs* PX) pathways, as revealed by the action of Stau, PD and U126, respectively, which were added to the cultures (Figure 6B). We confirmed that the inhibitors alone did not modify cell viability (data not shown).

Apoptosis is a cell death mechanism that could improve antitumor actions of chemotherapy, and the activation of protein kinases is frequently associated with the activation of pro/anti-apoptotic signaling pathways. As expected, we found a reduction in cell viability due to the apoptosis induced by PX (PX: 16.7% ± 1.4%; control: 6.1% ± 0.7%, *P* < 0.001). The presence of NIC reduced the effect of PX. The percentage of apoptotic cells was 9.3 ± 0.6 (*P* < 0.05 *vs* control or *P* < 0.001 *vs* PX) (Figure 7). We also observed that the effect of NIC on PX-treated cells was totally reversed in the presence of nicotinic antagonists (*P* > 0.05 *vs* PX) (Figure 7).

An important aspect in cancer chemotherapy is the development of resistance. This phenomenon has been linked to several proteins, including ABCG2, which is a drug extrusion pump that decreases the effectiveness of antitumor drugs. Thus, we next analyzed ABCG2 expression by Western blot. As shown in Figure 8, MDA-MB-231 cells expressed this protein and the addition of PX caused a significant increase in its expression (*P* < 0.05). The presence of NIC during PX treatment potently increased ABCG2 levels in tumor cells (*P* < 0.001 *vs* PX). The pretreatment of cells with nicotinic antagonists reduced NIC action on ABCG2 expression (*P* > 0.05 *vs* PX+NIC) (Figure 8).

**DISCUSSION**

Our results reveal the mechanisms by which NIC decreases the cytotoxic effects of PX on human TN breast cancer MDA-MB-231 cells. Several authors have described the effect of nAChR activation on cell proliferation. Regarding the latter, NIC from tobacco smoke stimulates nAChRs expressed in the oral cavity[49], esophagus[50], stomach[51], intestine[52] and lungs[53-55]. NIC can also trigger malignant transformation in smoking patients and increases the risk of developing lung cancer[56,57]. Less is known about the effects of NIC on developing tumors or promoting malignant growth in other organs near the lung that also express nAChRs, such as is the case of the breast. Previous studies have shown that human breast tumors express the α7 and α9 nAChR subtypes[58,59]. In the present study, we confirmed that these receptors are functional in MDA-MB-231 tumor cells as treatment with NIC increased cell viability in a concentration-dependent manner. This effect was reversed by the pre-treatment of cells with selective nicotinic antagonists.

Previous reports have indicated that the presence of NIC (due to smoking) reduces the effectiveness of chemotherapy in lung cancer patients[60]. Less evidence is available about the effect of NIC on breast cancer patients during chemotherapy administration. PX is a first-choice drug in breast cancer treatment due to its antimitotic ability and its effect on inhibiting different tumor progression pathways[61]. Our results confirm that PX acts on MDA-MB-231 tumor cells by reducing their viability in a dose-dependent manner. The presence of NIC at a concentration similar to that present in the blood of passive smokers[62,63] reduced the potency of PX by more than one order of magnitude. It is also important to highlight that the administration of NIC is effective in reducing the PX effect in other TN tumor cells such as MDA-MB-468, and in luminal A MCF-7 tumor cells. In a human gastric cancer model, Tu *et al*[64] observed a reduction in the effect of PX on cell viability due to NIC through the activation of α7 nAChRs. Similarly, here we demonstrated that both the α7 and α9 nAChR subtypes are involved in this effect, a fact also evident when PX chemotherapy was administered in cycles to TN breast tumor cells.

Several authors have demonstrated that chemotherapeutic drugs control tumor tissue growth by reducing cell viability[28,65,66] and activating distinct signaling transduction pathways that involve PKC[67], MEK[68], ERK1/2[69], Ras[70], p38MAPK[71] and NF-κB[72]. In particular, PX exerts its effects *via* the activation of different kinase signaling pathways depending on the cell type analyzed[73-75]. In our model, PX reduced cell viability through activation of the Ras, p38MAPK and NF-κB pathways. These results are in line with those obtained by Lu *et al*[76], who described that treatment with PX decreases ovarian carcinoma cell viability by activating the p38MAPK pathway, as well as with those of Okano and Rustgi[77], who observed that the treatment of human esophageal squamous cancer cells with PX increases cell death through the activation of Ras.

In the present study, when we analyzed signaling pathways involved in PX treatment in the presence of NIC, we determined that PKC, MEK and ERK1/2 also participate in the reduction of breast tumor cell viability. These results are similar to those of Chernyavsky *et al*[78], who described that, in human corneal epithelial cells, the stimulation of α7 nAChRs can activate the PKC-MEK-ERK1/2 signaling pathway through an increase in intracellular calcium, which induces an up-regulation of E cadherin expression related with corneal re-epithelization. In the same line of evidence, Wang *et al*[79] described that the activation of α7 nAChRs can mediate the proliferation of hepatocellular carcinoma through a TRAF6/NF-κB-dependent mechanism similar to that observed in the present study. Our results and those of others thus indicate the presence of antagonistic effects between PX and NIC on cell viability.

PX reduces breast cell viability, at least in part, through the induction of apoptosis[71]. We confirmed that this effect also occurs in our model as an increase in MDA-MB-231 cell apoptosis was observed after 48 h of treatment. The effect of PX on cellular apoptosis was attenuated by the activation of α7 and α9 nAChRs with NIC.

A frequent undesirable effect of antitumor therapy is innate or acquired resistance. In particular, PX can induce acquired resistance[80,81] by the transactivation of signaling pathways[82] or by triggering the overexpression of several proteins[83,84]. Considering the latter, the ABCG2 transporter plays an important role in the generation of resistance to PX treatment in breast adenocarcinomas[85]. In the present study, PX induced an increase in ABCG2 expression and this effect was potentiated by α7 and α9 nAChR activation by NIC. The increase in ABCG2 expression should lead to a higher rate of PX extrusion, partly explaining the reduction in the cytostatic effect of PX in the presence of NIC, as well as the decrease in the sensitivity to PX when administered in cycles in the presence of NIC. Similar results have been obtained by Mukherjee *et al*[86] and Amawi *et al*[87], who described that PX treatment increases the activation and expression of ABCG2 in different breast cancer cell lines and that this factor could mediate the resistance to treatment[88]. Moreover, Nimmakayala *et al*[89] and An *et al*[90] observed that the exposure of pancreatic and lung tumor cells to cigarette smoke caused an increase in the expression of this pump.

A possible mechanism of action of PX treatment in the presence of NIC in a TN tumor-bearing smoking patient is proposed in Figure 9.

**CONCLUSION**

Our results demonstrate that NIC at a concentration similar to that present in passive smokers' plasma can negatively modulate the cytotoxic/apoptotic effect of PX in TN breast tumors. The reduction in the sensitivity to PX could be due to an increase in the expression of the ABCG2 transporter in malignant cells. Additionally, our findings demonstrated the participation of different kinases and the NF-κB pathway, which would modulate cell viability in this effect. This information could allow the development of better strategies to improve TN breast cancer therapy, such as blocking nAChRs together with PX during chemotherapy administration to passive smoking patients.

**ARTICLE HIGHLIGHTS**

***Research background***

Triple negative is the subtype of breast cancer with the worst prognosis, showing an increase in resistance to chemotherapy in smoking patients, who have high levels of nicotine in their blood. In lung cancer, it has been proposed that the activation of nicotinic acetylcholine receptors could be responsible for the modulation of several parameters of tumor biology and the loss of effectiveness of chemotherapeutic treatment, but it is not known what occurs in a nearby organ such as the breast.

***Research motivation***

Given that breast tumor-bearing patients have a low efficiency to antitumor therapy, knowledge of the signaling pathways involved in this phenomenon is important to generate new therapeutic targets that improve sensitivity to treatment.

***Research objectives***

This research aimed to determine the signaling pathways involved in the nicotinic modulation of the cytostatic effect of paclitaxel in human triple negative breast cancer cells.

***Research methods***

The modulatory effect of nicotine on paclitaxel treatment was assessed by the 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The trypan blue exclusion assay was used to evaluate viable cells in response to different treatments. Protein expression levels were evaluated by Western blot assays and apoptosis was determined using immunofluorescence assays with annexin V and 7AAD.

***Research results***

Nicotine decreased paclitaxel’s inhibition of viability and apoptosis in MDA-MB-231 breast cancer cells. This modulation of viability is mediated by the activation of α7 and α9 nicotinic acetylcholine receptors and protein kinases PKC, Ras, MEK, ERK, p38MAPK and the NF-κB pathway. Cells surviving paclitaxel treatment in the presence of nicotine are less sensitive to another cycle with the chemotherapeutic agent probably due an increase in the protein expression of ATP binding cassette transporter G2.

***Research conclusions***

Nicotine modulates the cytotoxic/apoptotic effects of paclitaxel and knowledge of its signaling pathway mediators could allow the development of better strategies to improve triple negative breast cancer therapy, such as nicotinic acetylcholine receptors blockage together with paclitaxel during chemotherapy administration to smoking patients.

***Research perspectives***

Knowledge of the mediators that participate in the nicotinic modulation of paclitaxel’s effect will allow the development of new antitumor strategies that could be applied not only to other subtypes of mammary tumors, but also to tumors in other organs of smoking patients.

**REFERENCES**

1 **Paumgartten FJR**, Gomes-Carneiro MR, Oliveira ACAX. The impact of tobacco additives on cigarette smoke toxicity: a critical appraisal of tobacco industry studies. *Cad Saude Publica* 2017; **33Suppl 3**: e00132415 [PMID: 28954055 DOI: 10.1590/0102-311X00132415]

2 **DiSilvio B**, Baqdunes M, Alhajhusain A, Cheema T. Smoking Addiction and Strategies for Cessation. *Crit Care Nurs Q* 2021; **44**: 33-48 [PMID: 33234858 DOI: 10.1097/CNQ.0000000000000338]

3 **Wittenberg RE**, Wolfman SL, De Biasi M, Dani JA. Nicotinic acetylcholine receptors and nicotine addiction: A brief introduction. *Neuropharmacology* 2020; **177**: 108256 [PMID: 32738308 DOI: 10.1016/j.neuropharm.2020.108256]

4 **Sine SM**, Engel AG. Recent advances in Cys-loop receptor structure and function. *Nature* 2006; **440**: 448-455 [PMID: 16554804 DOI: 10.1038/nature04708]

5 **Gharpure A**, Noviello CM, Hibbs RE. Progress in nicotinic receptor structural biology. *Neuropharmacology* 2020; **171**: 108086 [PMID: 32272141 DOI: 10.1016/j.neuropharm.2020.108086]

6 **Schuller HM**. Cell type specific, receptor-mediated modulation of growth kinetics in human lung cancer cell lines by nicotine and tobacco-related nitrosamines. *Biochem Pharmacol* 1989; **38**: 3439-3442 [PMID: 2573356 DOI: 10.1016/0006-2952(89)90112-3]

7 **Diabasana Z**, Perotin JM, Belgacemi R, Ancel J, Mulette P, Delepine G, Gosset P, Maskos U, Polette M, Deslée G, Dormoy V. Nicotinic Receptor Subunits Atlas in the Adult Human Lung. *Int J Mol Sci* 2020; **21** [PMID: 33050277 DOI: 10.3390/ijms21207446]

8 **McArdle S**, Garg LC. Cholinergic stimulation of phosphoinositide hydrolysis in renal medullary collecting duct cells. *J Pharmacol Exp Ther* 1989; **248**: 682-686 [PMID: 2537409]

9 **Zheng CM**, Lee YH, Chiu IJ, Chiu YJ, Sung LC, Hsu YH, Chiu HW. Nicotine Causes Nephrotoxicity through the Induction of NLRP6 Inflammasome and Alpha7 Nicotinic Acetylcholine Receptor. *Toxics* 2020; **8** [PMID: 33114531 DOI: 10.3390/toxics8040092]

10 **Frieling T**, Cooke HJ, Wood JD. Synaptic transmission in submucosal ganglia of guinea pig distal colon. *Am J Physiol* 1991; **260**: G842-G849 [PMID: 1647674 DOI: 10.1152/ajpgi.1991.260.6.G842]

11 **Ten Hove AS**, Brinkman DJ, Li Yim AYF, Verseijden C, Hakvoort TBM, Admiraal I, Welting O, van Hamersveld PHP, Sinniger V, Bonaz B, Luyer MD, de Jonge WJ. The role of nicotinic receptors in SARS-CoV-2 receptor ACE2 expression in intestinal epithelia. *Bioelectron Med* 2020; **6**: 20 [PMID: 33123616 DOI: 10.1186/s42234-020-00057-1]

12 **Chen ZB**, Liu C, Chen FQ, Li SY, Liang Q, Liu LY. Effects of tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) on the activation of ERK1/2 MAP kinases and the proliferation of human mammary epithelial cells. *Environ Toxicol Pharmacol* 2006; **22**: 283-291 [PMID: 21783722 DOI: 10.1016/j.etap.2006.04.001]

13 **Sun Z**, Bao J, Zhangsun M, Dong S, Zhangsun D, Luo S. αO-Conotoxin GeXIVA Inhibits the Growth of Breast Cancer Cells *via* Interaction with α9 Nicotine Acetylcholine Receptors. *Mar Drugs* 2020; **18** [PMID: 32272701 DOI: 10.3390/md18040195]

14 **Shen T**, Le W, Yee A, Kamdar O, Hwang PH, Upadhyay D. Nicotine induces resistance to chemotherapy in nasal epithelial cancer. *Am J Rhinol Allergy* 2010; **24**: e73-e77 [PMID: 20338106 DOI: 10.2500/ajra.2010.24.3456]

15 **Boo HJ**, Min HY, Jang HJ, Yun HJ, Smith JK, Jin Q, Lee HJ, Liu D, Kweon HS, Behrens C, Lee JJ, Wistuba II, Lee E, Hong WK, Lee HY. The tobacco-specific carcinogen-operated calcium channel promotes lung tumorigenesis *via* IGF2 exocytosis in lung epithelial cells. *Nat Commun* 2016; **7**: 12961 [PMID: 27666821 DOI: 10.1038/ncomms12961]

16 **Martínez AK**, Jensen K, Hall C, O'Brien A, Ehrlich L, White T, Meng F, Zhou T, Greene J Jr, Bernuzzi F, Invernizzi P, Dostal DE, Lairmore T, Alpini G, Glaser SS. Nicotine Promotes Cholangiocarcinoma Growth in Xenograft Mice. *Am J Pathol* 2017; **187**: 1093-1105 [PMID: 28315314 DOI: 10.1016/j.ajpath.2017.01.011]

17 **Schaal C**, Padmanabhan J, Chellappan S. The Role of nAChR and Calcium Signaling in Pancreatic Cancer Initiation and Progression. *Cancers (Basel)* 2015; **7**: 1447-1471 [PMID: 26264026 DOI: 10.3390/cancers7030845]

18 **Resende RR**, Gomes KN, Adhikari A, Britto LR, Ulrich H. Mechanism of acetylcholine-induced calcium signaling during neuronal differentiation of P19 embryonal carcinoma cells in vitro. *Cell Calcium* 2008; **43**: 107-121 [PMID: 17662384 DOI: 10.1016/j.ceca.2007.04.007]

19 **Grando SA**. Connections of nicotine to cancer. *Nat Rev Cancer* 2014; **14**: 419-429 [PMID: 24827506 DOI: 10.1038/nrc3725]

20 **Schuller HM**. Neurotransmitter receptor-mediated signaling pathways as modulators of carcinogenesis. *Prog Exp Tumor Res* 2007; **39**: 45-63 [PMID: 17314500 DOI: 10.1159/000100045]

21 **Pernarella M**, Piovesana R, Matera C, Faroni A, Fiore M, Dini L, Reid AJ, Dallanoce C, Tata AM. Effects mediated by the α7 nicotinic acetylcholine receptor on cell proliferation and migration in rat adipose-derived stem cells. *Eur J Histochem* 2020; **64** [PMID: 33334089 DOI: 10.4081/ejh.2020.3159]

22 **Qi JC**, Xue WY, Zhang YP, Qu CB, Lu BS, Yin YW, Liu KL, Wang DB, Li W, Zhao ZM. Cholinergic α5 nicotinic receptor is involved in the proliferation and invasion of human prostate cancer cells. *Oncol Rep* 2020; **43**: 159-168 [PMID: 31789411 DOI: 10.3892/or.2019.7411]

23 **Youssef ME**, El-Mas MM, Abdelrazek HM, El-Azab MF. α7-nAChRs-mediated therapeutic angiogenesis accounts for the advantageous effect of low nicotine doses against myocardial infarction in rats. *Eur J Pharmacol* 2021; **898**: 173996 [PMID: 33684450 DOI: 10.1016/j.ejphar.2021.173996]

24 **Hsu CC**, Tsai KY, Su YF, Chien CY, Chen YC, Wu YC, Liu SY, Shieh YS. α7-Nicotine acetylcholine receptor mediated nicotine induced cell survival and cisplatin resistance in oral cancer. *Arch Oral Biol* 2020; **111**: 104653 [PMID: 31935534 DOI: 10.1016/j.archoralbio.2020.104653]

25 **Banerjee J**, Al-Wadei HA, Al-Wadei MH, Dagnon K, Schuller HM. Differential modulation of nicotine-induced gemcitabine resistance by GABA receptor agonists in pancreatic cancer cell xenografts and in vitro. *BMC Cancer* 2014; **14**: 725 [PMID: 25260978 DOI: 10.1186/1471-2407-14-725]

26 **Shimizu R**, Ibaragi S, Eguchi T, Kuwajima D, Kodama S, Nishioka T, Okui T, Obata K, Takabatake K, Kawai H, Ono K, Okamoto K, Nagatsuka H, Sasaki A. Nicotine promotes lymph node metastasis and cetuximab resistance in head and neck squamous cell carcinoma. *Int J Oncol* 2019; **54**: 283-294 [PMID: 30431077 DOI: 10.3892/ijo.2018.4631]

27 **Arribas RL**, Bordas A, Domènech Omella J, Cedillo JL, Janssens V, Montiel C, de Los Ríos C. An okadaic acid fragment analogue prevents nicotine-induced resistance to cisplatin by recovering PP2A activity in non-small cell lung cancer cells. *Bioorg Chem* 2020; **100**: 103874 [PMID: 32361056 DOI: 10.1016/j.bioorg.2020.103874]

28 **Li H**, Wang S, Takayama K, Harada T, Okamoto I, Iwama E, Fujii A, Ota K, Hidaka N, Kawano Y, Nakanishi Y. Nicotine induces resistance to erlotinib *via* cross-talk between α 1 nAChR and EGFR in the non-small cell lung cancer xenograft model. *Lung Cancer* 2015; **88**: 1-8 [PMID: 25670150 DOI: 10.1016/j.lungcan.2015.01.017]

29 **Cheng WL**, Chen KY, Lee KY, Feng PH, Wu SM. Nicotinic-nAChR signaling mediates drug resistance in lung cancer. *J Cancer* 2020; **11**: 1125-1140 [PMID: 31956359 DOI: 10.7150/jca.36359]

30 **Czyżykowski R**, Połowinczak-Przybyłek J, Potemski P. Nicotine-induced resistance of non-small cell lung cancer to treatment--possible mechanisms. *Postepy Hig Med Dosw (Online)* 2016; **70**: 186-193 [PMID: 26943316 DOI: 10.5604/17322693.1196391]

31 **Imabayashi T**, Uchino J, Osoreda H, Tanimura K, Chihara Y, Tamiya N, Kaneko Y, Yamada T, Takayama K. Nicotine Induces Resistance to Erlotinib Therapy in Non-Small-Cell Lung Cancer Cells Treated with Serum from Human Patients. *Cancers (Basel)* 2019; **11** [PMID: 30818860 DOI: 10.3390/cancers11030282]

32 **Togashi Y**, Hayashi H, Okamoto K, Fumita S, Terashima M, de Velasco MA, Sakai K, Fujita Y, Tomida S, Nakagawa K, Nishio K. Chronic nicotine exposure mediates resistance to EGFR-TKI in EGFR-mutated lung cancer *via* an EGFR signal. *Lung Cancer* 2015; **88**: 16-23 [PMID: 25704955 DOI: 10.1016/j.lungcan.2015.01.027]

33 **Li G**, Xu D, Sun J, Zhao S, Zheng D. Paclitaxel inhibits proliferation and invasion and promotes apoptosis of breast cancer cells by blocking activation of the PI3K/AKT signaling pathway. *Adv Clin Exp Med* 2020; **29**: 1337-1345 [PMID: 33269821 DOI: 10.17219/acem/127681]

34 **Zhang Y**. International agency for research on cancer (iarc). In: Zhang Y. Encyclopedia of global health. Thousand Oaks, CA: SAGE Publications, Inc., 2008: 928-928

35 **Cheang MC**, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK, Perou CM, Nielsen TO. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res* 2008; **14**: 1368-1376 [PMID: 18316557 DOI: 10.1158/1078-0432.CCR-07-1658]

36 **Cheang MC**, Chia SK, Voduc D, Gao D, Leung S, Snider J, Watson M, Davies S, Bernard PS, Parker JS, Perou CM, Ellis MJ, Nielsen TO. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. *J Natl Cancer Inst* 2009; **101**: 736-750 [PMID: 19436038 DOI: 10.1093/jnci/djp082]

37 **He L**, Lv Y, Song Y, Zhang B. The prognosis comparison of different molecular subtypes of breast tumors after radiotherapy and the intrinsic reasons for their distinct radiosensitivity. *Cancer Manag Res* 2019; **11**: 5765-5775 [PMID: 31303789 DOI: 10.2147/CMAR.S213663]

38 **Ramaswamy B**, Puhalla S. Docetaxel: a tubulin-stabilizing agent approved for the management of several solid tumors. *Drugs Today (Barc)* 2006; **42**: 265-279 [PMID: 16703123 DOI: 10.1358/dot.2006.42.4.968648]

39 **Weaver BA**. How Taxol/paclitaxel kills cancer cells. *Mol Biol Cell* 2014; **25**: 2677-2681 [PMID: 25213191 DOI: 10.1091/mbc.E14-04-0916]

40 **Alqahtani FY**, Aleanizy FS, El Tahir E, Alkahtani HM, AlQuadeib BT. Paclitaxel. *Profiles Drug Subst Excip Relat Methodol* 2019; **44**: 205-238 [PMID: 31029218 DOI: 10.1016/bs.podrm.2018.11.001]

41 **Chen TR**. In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. *Exp Cell Res* 1977; **104**: 255-262 [PMID: 65285 DOI: 10.1016/0014-4827(77)90089-1]

42 **Bradford MM**. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248-254 [PMID: 942051 DOI: 10.1006/abio.1976.9999]

43 **Español AJ**, Sales ME. Different muscarinc receptors are involved in the proliferation of murine mammary adenocarcinoma cell lines. *Int J Mol Med* 2004; **13**: 311-317 [PMID: 14719140]

44 **Davel LE**, Rimmaudo L, Español A, de la Torre E, Jasnis MA, Ribeiro ML, Gotoh T, de Lustig ES, Sales ME. Different mechanisms lead to the angiogenic process induced by three adenocarcinoma cell lines. *Angiogenesis* 2004; **7**: 45-51 [PMID: 15302995 DOI: 10.1023/B:AGEN.0000037329.45326.a8]

45 **Curtis MJ**, Alexander S, Cirino G, Docherty JR, George CH, Giembycz MA, Hoyer D, Insel PA, Izzo AA, Ji Y, MacEwan DJ, Sobey CG, Stanford SC, Teixeira MM, Wonnacott S, Ahluwalia A. Experimental design and analysis and their reporting II: updated and simplified guidance for authors and peer reviewers. *Br J Pharmacol* 2018; **175**: 987-993 [PMID: 29520785 DOI: 10.1111/bph.14153]

46 **Zil-a-Rubab**, Ata-ur-Rahman M. Estimation of serum nicotine by gas chromatography in smokers, passive smokers and never smokers. *J Pak Med Assoc* 2012; **62**: 790-793 [PMID: 23862251]

47 **Kamiyama M**, Naguro I, Ichijo H. [Functional Analysis of Apoptosis Signal-regulating Kinase Family in a Murine Model of Tumor Metastasis]. *Yakugaku Zasshi* 2019; **139**: 743-751 [PMID: 31061344 DOI: 10.1248/yakushi.18-00185-2]

48 **Han YB**, Yang JM, Kwon HJ, Lee JO, Lee JS, Paik JH. Clinicopathologic and Prognostic Significance of Bruton's Tyrosine Kinase Expression in Diffuse Large B-Cell Lymphoma. *Anticancer Res* 2021; **41**: 5677-5692 [PMID: 34732441 DOI: 10.21873/anticanres.15384]

49 **Nishioka T**, Tada H, Ibaragi S, Chen C, Sasano T. Nicotine exposure induces the proliferation of oral cancer cells through the α7 subunit of the nicotinic acetylcholine receptor. *Biochem Biophys Res Commun* 2019; **509**: 514-520 [PMID: 30598264 DOI: 10.1016/j.bbrc.2018.12.154]

50 **Li N**, Liu K, Dong S, Ou L, Li J, Lai M, Wang Y, Bao Y, Shi H, Wang X, Wang S. Identification of CHRNB4 as a Diagnostic/Prognostic Indicator and Therapeutic Target in Human Esophageal Squamous Cell Carcinoma. *Front Oncol* 2020; **10**: 571167 [PMID: 33304845 DOI: 10.3389/fonc.2020.571167]

51 **Bu X**, Yin C, Zhang X, Zhang A, Shao X, Zhang Y, Yan Y. LaSota Strain Expressing The Rabies Virus Glycoprotein (rL-RVG) Suppresses Gastric Cancer by Inhibiting the Alpha 7 Nicotinic Acetylcholine Receptor (α7 nAChR)/Phosphoinositide 3-Kinase (PI3K)/AKT Pathway. *Med Sci Monit* 2019; **25**: 5482-5492 [PMID: 31337746 DOI: 10.12659/MSM.915251]

52 **Xiang T**, Fei R, Wang Z, Shen Z, Qian J, Chen W. Nicotine enhances invasion and metastasis of human colorectal cancer cells through the nicotinic acetylcholine receptor downstream p38 MAPK signaling pathway. *Oncol Rep* 2016; **35**: 205-210 [PMID: 26530054 DOI: 10.3892/or.2015.4363]

53 **Witayateeraporn W**, Arunrungvichian K, Pothongsrisit S, Doungchawee J, Vajragupta O, Pongrakhananon V. α7-Nicotinic acetylcholine receptor antagonist QND7 suppresses non-small cell lung cancer cell proliferation and migration *via* inhibition of Akt/mTOR signaling. *Biochem Biophys Res Commun* 2020; **521**: 977-983 [PMID: 31727368 DOI: 10.1016/j.bbrc.2019.11.018]

54 **Sitapara RA**, Gauthier AG, Valdés-Ferrer SI, Lin M, Patel V, Wang M, Martino AT, Perron JC, Ashby CR Jr, Tracey KJ, Pavlov VA, Mantell LL. The α7 nicotinic acetylcholine receptor agonist, GTS-21, attenuates hyperoxia-induced acute inflammatory lung injury by alleviating the accumulation of HMGB1 in the airways and the circulation. *Mol Med* 2020; **26**: 63 [PMID: 32600307 DOI: 10.1186/s10020-020-00177-z]

55 **Qian J**, Liu YQ, Sun ZH, Zhangsun DT, Luo SL. Identification of nicotinic acetylcholine receptor subunits in different lung cancer cell lines and the inhibitory effect of alpha-conotoxin TxID on lung cancer cell growth. *Eur J Pharmacol* 2019; **865**: 172674 [PMID: 31634461 DOI: 10.1016/j.ejphar.2019.172674]

56 **Tonini G**, D'Onofrio L, Dell'Aquila E, Pezzuto A. New molecular insights in tobacco-induced lung cancer. *Future Oncol* 2013; **9**: 649-655 [PMID: 23647294 DOI: 10.2217/fon.13.32]

57 **González-Marrón A**, Martín-Sánchez JC, Garcia-Alemany F, Martínez-Martín E, Matilla-Santander N, Cartanyà-Hueso À, Vidal C, García M, Martínez-Sánchez JM. Estimation of the Risk of Lung Cancer in Women Participating in a Population-Based Breast Cancer Screening Program. *Arch Bronconeumol (Engl Ed)* 2020; **56**: 277-281 [PMID: 31629546 DOI: 10.1016/j.arbres.2019.04.014]

58 **Aali N**, Motalleb G. The effect of nicotine on the expressions of the α7 nicotinic receptor gene and Bax and Bcl-2 proteins in the mammary gland epithelial-7 breast cancer cell line and its relationship to drug resistance. *Cell Mol Biol Lett* 2015; **20**: 948-964 [PMID: 26812869 DOI: 10.1515/cmble-2015-0056]

59 **Clarke MR**, Jones B, Squires CLM, Imhoff FM, Harwood DT, Rhodes L, Selwood AI, McNabb PS, Baird SK. Cyclic Imine Pinnatoxin G is Cytotoxic to Cancer Cell Lines *via* Nicotinic Acetylcholine Receptor-Driven Classical Apoptosis. *J Nat Prod* 2021; **84**: 2035-2042 [PMID: 34170700 DOI: 10.1021/acs.jnatprod.1c00418]

60 **Zhang J**, Kamdar O, Le W, Rosen GD, Upadhyay D. Nicotine induces resistance to chemotherapy by modulating mitochondrial signaling in lung cancer. *Am J Respir Cell Mol Biol* 2009; **40**: 135-146 [PMID: 18676776 DOI: 10.1165/rcmb.2007-0277OC]

61 **Abu Samaan TM**, Samec M, Liskova A, Kubatka P, Büsselberg D. Paclitaxel's Mechanistic and Clinical Effects on Breast Cancer. *Biomolecules* 2019; **9** [PMID: 31783552 DOI: 10.3390/biom9120789]

62 **García Calzado MC**, García Rojas JF, Mangas Rojas A, Martínez Izquierdo D, Repetto M, Millán J. [Tobacco and arterial pressure (I.). The hormonal changes in a model of acute nicotine overload]. *An Med Interna* 1990; **7**: 340-344 [PMID: 1966467]

63 **Lindell G**, Farnebo LO, Chen D, Nexø E, Rask Madsen J, Bukhave K, Graffner H. Acute effects of smoking during modified sham feeding in duodenal ulcer patients. An analysis of nicotine, acid secretion, gastrin, catecholamines, epidermal growth factor, prostaglandin E2, and bile acids. *Scand J Gastroenterol* 1993; **28**: 487-494 [PMID: 8322024 DOI: 10.3109/00365529309098254]

64 **Tu CC**, Huang CY, Cheng WL, Hung CS, Uyanga B, Wei PL, Chang YJ. The α7-nicotinic acetylcholine receptor mediates the sensitivity of gastric cancer cells to taxanes. *Tumour Biol* 2016; **37**: 4421-4428 [PMID: 26499946 DOI: 10.1007/s13277-015-4260-y]

65 **Duarte D**, Vale N. New Trends for Antimalarial Drugs: Synergism between Antineoplastics and Antimalarials on Breast Cancer Cells. *Biomolecules* 2020; **10** [PMID: 33271968 DOI: 10.3390/biom10121623]

66 **Pereira DG**, Rendeiro MM, Cortes VF, Barbosa LA, Quintas LEM. Antagonistic anticancer effect of paclitaxel and digoxin combination. *J Cell Biochem* 2019; **120**: 13107-13114 [PMID: 30883884 DOI: 10.1002/jcb.28583]

67 **Dey A**, Islam SMA, Patel R, Acevedo-Duncan M. The interruption of atypical PKC signaling and Temozolomide combination therapy against glioblastoma. *Cell Signal* 2021; **77**: 109819 [PMID: 33147518 DOI: 10.1016/j.cellsig.2020.109819]

68 **Yao W**, Lin Z, Shi P, Chen B, Wang G, Huang J, Sui Y, Liu Q, Li S, Lin X, Liu Q, Yao H. Delicaflavone induces ROS-mediated apoptosis and inhibits PI3K/AKT/mTOR and Ras/MEK/Erk signaling pathways in colorectal cancer cells. *Biochem Pharmacol* 2020; **171**: 113680 [PMID: 31669234 DOI: 10.1016/j.bcp.2019.113680]

69 **Yu R**, Wang M, Wang M, Han L. Melittin suppresses growth and induces apoptosis of non-small-cell lung cancer cells *via* down-regulation of TGF-β-mediated ERK signal pathway. *Braz J Med Biol Res* 2020; **54**: e9017 [PMID: 33331417 DOI: 10.1590/1414-431X20209017]

70 **Lu H**, Zhou L, Zuo H, Le W, Hu J, Zhang T, Li M, Yuan Y. Overriding sorafenib resistance *via* blocking lipid metabolism and Ras by sphingomyelin synthase 1 inhibition in hepatocellular carcinoma. *Cancer Chemother Pharmacol* 2021; **87**: 217-228 [PMID: 33226447 DOI: 10.1007/s00280-020-04199-6]

71 **Sun WL**, Wang L, Luo J, Zhu HW, Cai ZW. Ambra1 inhibits paclitaxel-induced apoptosis in breast cancer cells by modulating the Bim/mitochondrial pathway. *Neoplasma* 2019; **66**: 377-385 [PMID: 30784282 DOI: 10.4149/neo\_2018\_180710N467]

72 **Liu YK**, Jia YJ, Liu SH, Ma J. FSTL1 increases cisplatin sensitivity in epithelial ovarian cancer cells by inhibition of NF-κB pathway. *Cancer Chemother Pharmacol* 2021; **87**: 405-414 [PMID: 33392640 DOI: 10.1007/s00280-020-04215-9]

73 **Jung M**, Park KH, Kim HM, Kim TS, Zhang X, Park SM, Beom SH, Kim HS, Cheong JH, Chung HC, Soong J, Lin SC, Rha SY. Inhibiting casein kinase 2 overcomes paclitaxel resistance in gastric cancer. *Gastric Cancer* 2019; **22**: 1153-1163 [PMID: 31098863 DOI: 10.1007/s10120-019-00971-7]

74 **Huang T**, Yang L, Wang G, Ding G, Peng B, Wen Y, Wang Z. Inhibition of Skp2 sensitizes lung cancer cells to paclitaxel. *Onco Targets Ther* 2017; **10**: 439-446 [PMID: 28176922 DOI: 10.2147/OTT.S125789]

75 **Furuse J**, Nagashima F. Emerging protein kinase inhibitors for treating pancreatic cancer. *Expert Opin Emerg Drugs* 2017; **22**: 77-86 [PMID: 28253828 DOI: 10.1080/14728214.2017.1293648]

76 **Lu M**, Xiao L, Li Z. The relationship between p38MAPK and apoptosis during paclitaxel resistance of ovarian cancer cells. *J Huazhong Univ Sci Technolog Med Sci* 2007; **27**: 725-728 [PMID: 18231754 DOI: 10.1007/s11596-007-0628-6]

77 **Okano J**, Rustgi AK. Paclitaxel induces prolonged activation of the Ras/MEK/ERK pathway independently of activating the programmed cell death machinery. *J Biol Chem* 2001; **276**: 19555-19564 [PMID: 11278851 DOI: 10.1074/jbc.M011164200]

78 **Chernyavsky AI**, Galitovskiy V, Grando SA. Molecular mechanisms of synergy of corneal muscarinic and nicotinic acetylcholine receptors in upregulation of E-cadherin expression. *Int Immunopharmacol* 2015; **29**: 15-20 [PMID: 25957200 DOI: 10.1016/j.intimp.2015.04.036]

79 **Wang Y**, Zhou M, Shang D. Berberine inhibits human gastric cancer cell growth *via* deactivation of p38/JNK pathway, induction of mitochondrial-mediated apoptosis, caspase activation and NF-κB inhibition. *J BUON* 2020; **25**: 314-318 [PMID: 32277648]

80 **Dan VM**, Raveendran RS, Baby S. Resistance to Intervention: Paclitaxel in Breast Cancer. *Mini Rev Med Chem* 2021; **21**: 1237-1268 [PMID: 33319669 DOI: 10.2174/1389557520999201214234421]

81 **Zsóková E**, Ilkovičová L, Kimáková P, Fecková B, Solár P. Erythropoietin receptor induces a paclitaxel resistance phenotype in mammary adenocarcinoma cells. *Oncol Rep* 2019; **42**: 1149-1160 [PMID: 31322257 DOI: 10.3892/or.2019.7222]

82 **Chi Y**, Xue J, Huang S, Xiu B, Su Y, Wang W, Guo R, Wang L, Li L, Shao Z, Jin W, Wu Z, Wu J. CapG promotes resistance to paclitaxel in breast cancer through transactivation of PIK3R1/P50. *Theranostics* 2019; **9**: 6840-6855 [PMID: 31660072 DOI: 10.7150/thno.36338]

83 **Khongkow P**, Gomes AR, Gong C, Man EP, Tsang JW, Zhao F, Monteiro LJ, Coombes RC, Medema RH, Khoo US, Lam EW. Paclitaxel targets FOXM1 to regulate KIF20A in mitotic catastrophe and breast cancer paclitaxel resistance. *Oncogene* 2016; **35**: 990-1002 [PMID: 25961928 DOI: 10.1038/onc.2015.152]

84 **Lai H**, Wang R, Li S, Shi Q, Cai Z, Li Y, Liu Y. LIN9 confers paclitaxel resistance in triple negative breast cancer cells by upregulating CCSAP. *Sci China Life Sci* 2020; **63**: 419-428 [PMID: 31420851 DOI: 10.1007/s11427-019-9581-8]

85 **Němcová-Fürstová V**, Kopperová D, Balušíková K, Ehrlichová M, Brynychová V, Václavíková R, Daniel P, Souček P, Kovář J. Characterization of acquired paclitaxel resistance of breast cancer cells and involvement of ABC transporters. *Toxicol Appl Pharmacol* 2016; **310**: 215-228 [PMID: 27664577 DOI: 10.1016/j.taap.2016.09.020]

86 **Mukherjee P**, Gupta A, Chattopadhyay D, Chatterji U. Modulation of SOX2 expression delineates an end-point for paclitaxel-effectiveness in breast cancer stem cells. *Sci Rep* 2017; **7**: 9170 [PMID: 28835684 DOI: 10.1038/s41598-017-08971-2]

87 **Amawi H**, Sim HM, Tiwari AK, Ambudkar SV, Shukla S. ABC Transporter-Mediated Multidrug-Resistant Cancer. *Adv Exp Med Biol* 2019; **1141**: 549-580 [PMID: 31571174 DOI: 10.1007/978-981-13-7647-4\_12]

88 **Duz MB**, Karatas OF. Differential expression of ABCB1, ABCG2, and KLF4 as putative indicators for paclitaxel resistance in human epithelial type 2 cells. *Mol Biol Rep* 2021; **48**: 1393-1400 [PMID: 33506275 DOI: 10.1007/s11033-021-06167-6]

89 **Nimmakayala RK**, Seshacharyulu P, Lakshmanan I, Rachagani S, Chugh S, Karmakar S, Rauth S, Vengoji R, Atri P, Talmon GA, Lele SM, Smith LM, Thapa I, Bastola D, Ouellette MM, Batra SK, Ponnusamy MP. Cigarette Smoke Induces Stem Cell Features of Pancreatic Cancer Cells *via* PAF1. *Gastroenterology* 2018; **155**: 892-908.e6 [PMID: 29864419 DOI: 10.1053/j.gastro.2018.05.041]

90 **An Y**, Kiang A, Lopez JP, Kuo SZ, Yu MA, Abhold EL, Chen JS, Wang-Rodriguez J, Ongkeko WM. Cigarette smoke promotes drug resistance and expansion of cancer stem cell-like side population. *PLoS One* 2012; **7**: e47919 [PMID: 23144836 DOI: 10.1371/journal.pone.0047919]

**Footnotes**

**Institutional review board statement:** In the realization of the manuscript, no human samples or patients were used, so the authorization of the Institutional Review Board was not required.

**Institutional animal care and use committee statement:** In the realization of the manuscript, no animals were used, so the authorization of the Institutional Animal Care and Use Committee was not required.

**Conflict-of-interest statement:** The authors certify that they have no conflicts of interest (including but not limited to commercial, personal, political, intellectual or religious interests) for this article.

**Data sharing statement:** The technical appendix, statistical code, and dataset are available from the corresponding author at aespanol@fmed.uba.ar. Participants gave informed consent for data sharing.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review started:** December 27, 2021

**First decision:** February 15, 2022

**Article in press:**

**Specialty type:** Oncology

**Country/Territory of origin:** Argentina

**Peer-review report’s scientific quality classification**

Grade A (Excellent): A

Grade B (Very good): B

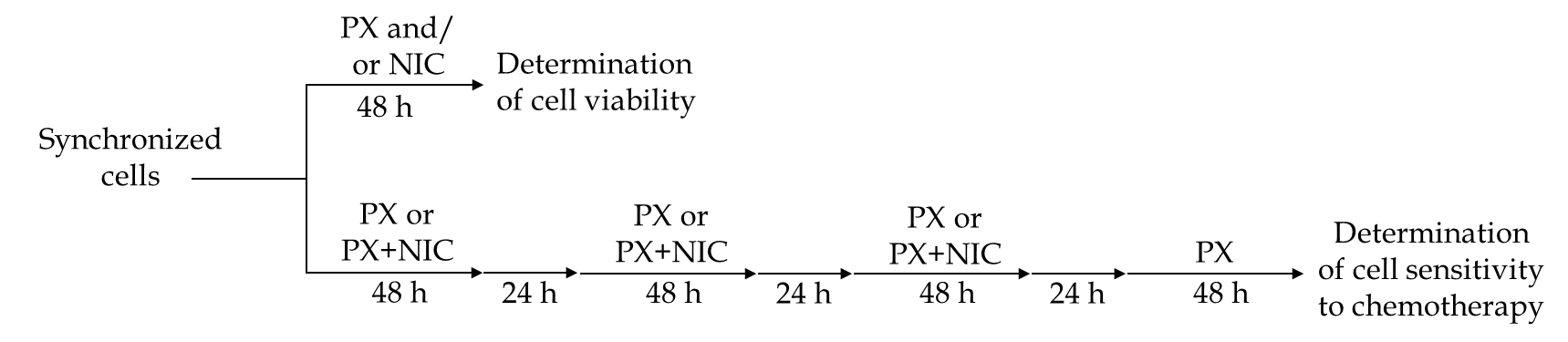
Grade C (Good): C

Grade D (Fair): 0

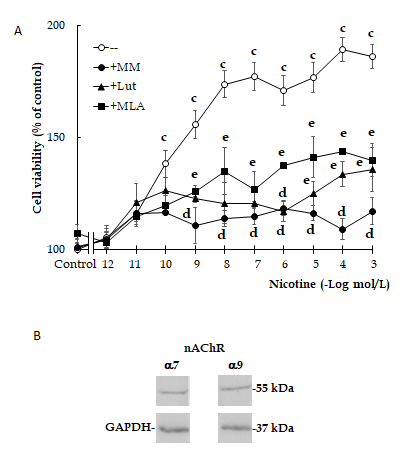
Grade E (Poor): 0

**P-Reviewer:** Khaled I, Egypt; Rossi T, Italy; Xie S, China **S-Editor:** Gao CC **L-Editor:** Webster JR **P-Editor:**

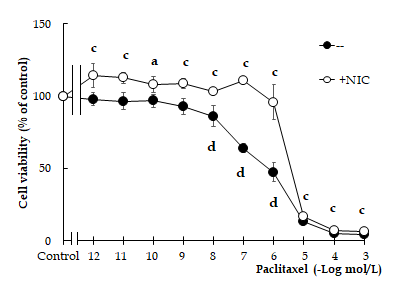
**Figure Legends**



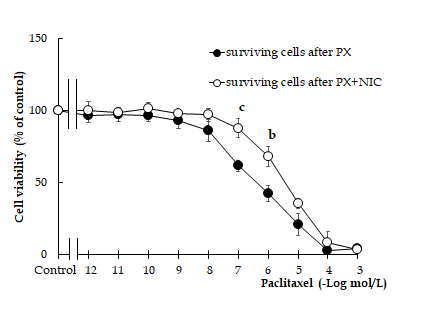
**Figure 1 Diagram of the administration schedule for the determination of cell viability or cell sensitivity to chemotherapy.** PX: Paclitaxel; NIC: Nicotine.



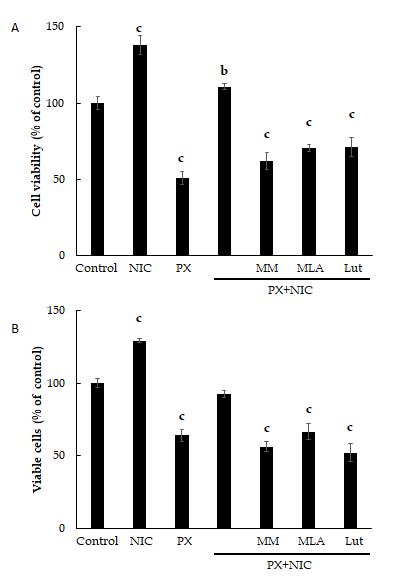
**Figure 2 MDA-MB-231 cell viability.** A: Concentration-response curves of nicotine on cell viability in the absence or presence of nicotinic antagonists: mecamylamine [non-selective for nicotinic acetylcholine receptors (nAChRs)], methyllycaconitine (selective for α7 nAChRs), or luteolin (selective for α9 nAChRs) at a concentration of 10-6 mol/L. Values are the mean ± SD of five experiments performed in duplicate. c*P* < 0.001 *vs* control; d*P* < 0.001 *vs* nicotine; e*P* < 0.001 *vs* control or nicotine; B: Western blot assay to detect α7 and α9 nAChR expression. Molecular weights are indicated on the right. The expression of glyceraldehyde 3-phosphate dehydrogenase was used as the loading control. One representative experiment of three is shown. MM: Mecamylamine; MLA: Methyllycaconitine; Lut: Luteolin; nAChRs: Nicotinic acetylcholine receptors; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.



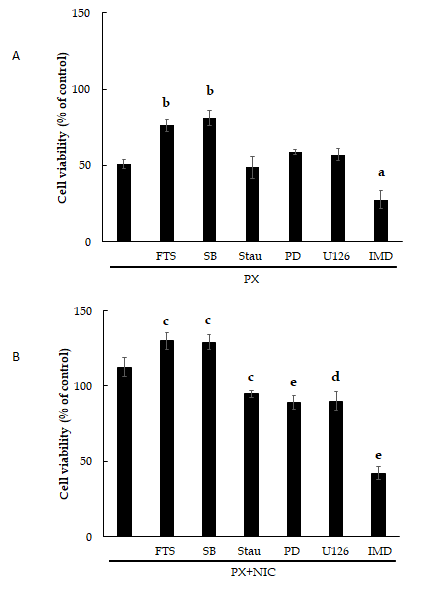
**Figure 3 MDA-MB-231 cell viability.** Concentration-response curves of paclitaxel on cell viability in the absence or presence of nicotine (NIC) (10-10 mol/L). Values are the mean ± SD of six experiments performed in duplicate. a*P* < 0.05; c*P* < 0.001 *vs* Control; d*P* < 0.001 *vs* +NIC. NIC: Nicotine.



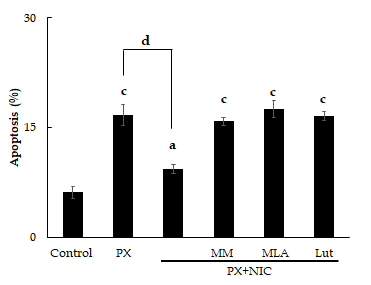
**Figure 4 Sensitivity of MDA-MB-231 cells to chemotherapy.** Concentration-response curves of paclitaxel (PX) on the viability of surviving cells after three cycles of PX treatment (10-7 mol/L) in the absence or presence of nicotine (10-10 mol/L). Values are the mean ± SD of three experiments performed in duplicate. b*P* < 0.01; c*P* < 0.001 *vs* surviving cells after three cycles of PX treatment. PX: Paclitaxel; NIC: Nicotine.



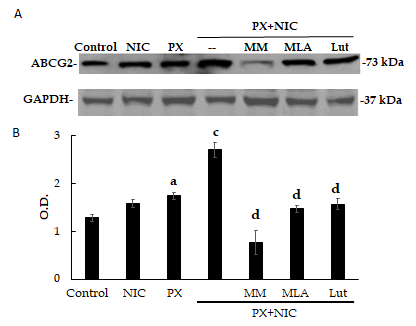
**Figure 5 Effect of nicotine on paclitaxel treatment.** A: Viability determination of MDA-MB-231 cells treated with nicotine (10-10 mol/L) and paclitaxel (10-7 mol/L) alone or in combination, in the absence or presence of nicotinic antagonists: mecamylamine [non-selective for nicotinic acetylcholine receptors (nAChRs)], methyllycaconitine (selective for α7 nAChRs), or luteolin (selective for α9 nAChRs) at a concentration of 10-6 mol/L; B: Determination of percentage of living MDA-MB-231 cells treated with the same drug combinations as those shown in Figure 5A. Values are the mean ± SD of four experiments performed in duplicate. b*P* < 0.01; c*P* < 0.001 *vs* control, considered as 100%. MM: Mecamylamine; MLA: Methyllycaconitine; Lut: Luteolin; PX: Paclitaxel; NIC: Nicotine.



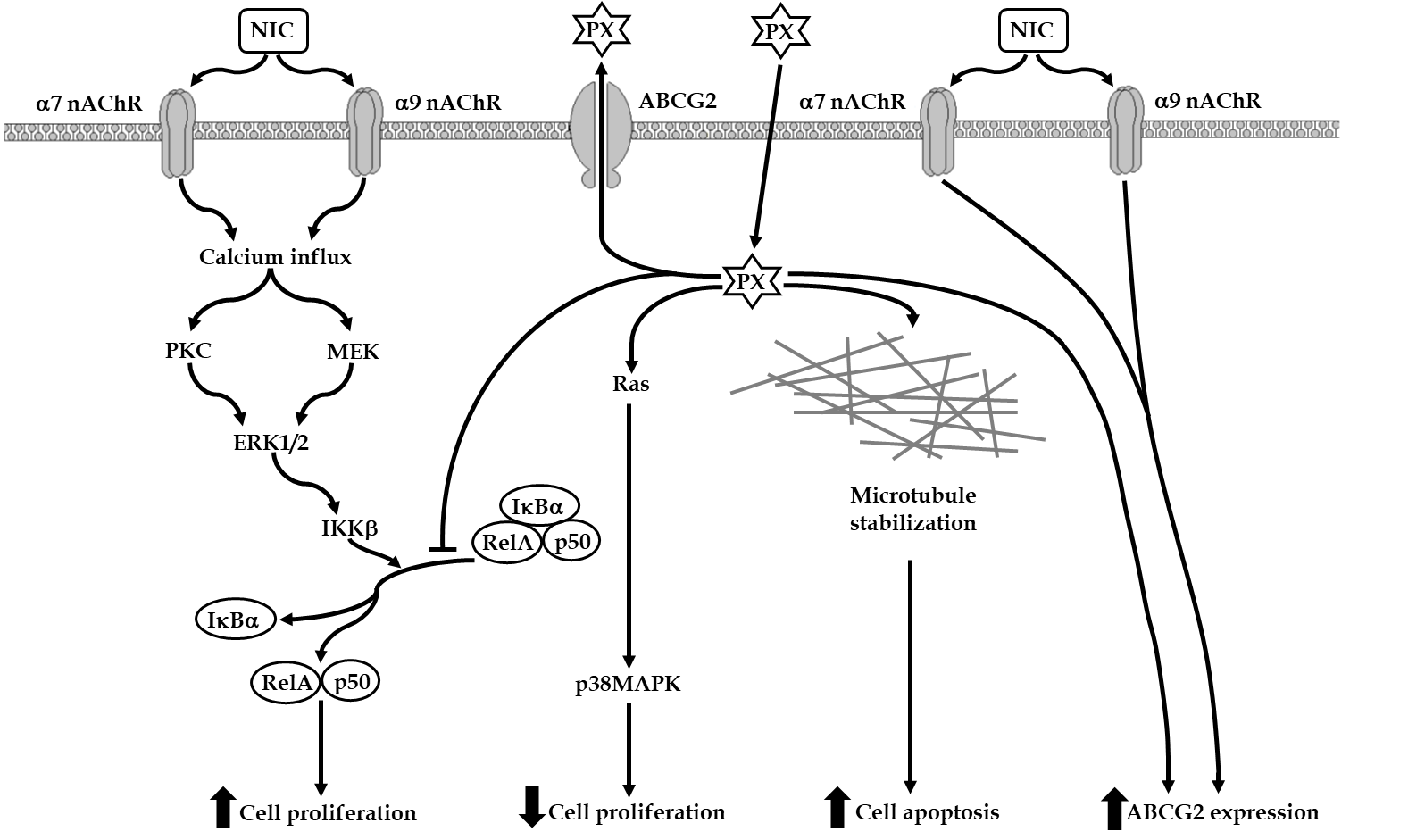
**Figure 6 Effect of paclitaxel and nicotine on MDA-MB-231 cell viability.** A: Cells were treated with paclitaxel (PX) (10-7 mol/L) and the mediators were evaluated in the absence or presence of the kinase inhibitors for: PKC (staurosporine, 10-8 mol/L), MEK (PD098059 PD, 10-5 mol/L), Ras (S-trans, trans-farnesylthiosalicylic acid, 10-6 mol/L), ERK1/2 (U126, 10-5 mol/L), p38MAPK (SB203580, 10-5 mol/L) or IKKβ (IMD354, 5 × 10-8 mol/L); B: Cells were treated with the combination of PX and nicotine (NIC) (10-10 mol/L) as well as with the same inhibitors as those shown in Figure 6A. Values are the mean ± SD of four experiments performed in duplicate. a*P* < 0.05; b*P* < 0.01 *vs* PX. c*P* < 0.05; d*P* < 0.01; e*P* < 0.001 *vs* PX+NIC. FTS: S-trans, trans-farnesylthiosalicylic acid; SB: SB203580; Stau: Staurosporine; PD: PD098059; PX: Paclitaxel; NIC: Nicotine.



**Figure 7 Effect of nicotine on paclitaxel-induced apoptosis in MDA-MB-231 cells.** Tumor cells were treated with paclitaxel (10-7 mol/L) in the absence or presence of the following nicotinic antagonists: mecamylamine [non-selective for nicotinic acetylcholine receptors (nAChRs)], methyllycaconitine (selective for α7 nAChRs), or luteolin (selective for α9 nAChRs) at a concentration of 10-6 mol/L. The percentage of apoptotic cells was determined by flow cytometry. Values are the mean ± SD of four experiments performed in duplicate. a*P* < 0.05; b*P* < 0.01; c*P* < 0.001 *vs* control; d*P* < 0.001. MM: Mecamylamine; MLA: Methyllycaconitine; Lut: Luteolin; PX: Paclitaxel; NIC: Nicotine.



**Figure 8 Effect of nicotine on paclitaxel-induced expression of ATP binding cassette transporter G2 protein in MDA-MB-231 cells.** A: ATP binding cassette transporter G2 expression was determined by Western blot assays in cells treated with paclitaxel (10-7 mol/L), nicotine (10-10 mol/L) or both, in the absence or presence of the nicotinic antagonists mecamylamine [non-selective for nicotinic acetylcholine receptors (nAChRs)], methyllycaconitine (selective for α7 nAChRs) or luteolin (selective for α9 nAChRs) at a concentration of 10-6 mol/L. Molecular weights are indicated on the right; B: The densitometric analysis of the bands is expressed as optical density units relative to the expression of glyceraldehyde 3-phosphate dehydrogenase protein used as the loading control. One representative experiment of three is shown. Values are the mean ± SD of three experiments. a*P* < 0.05; c*P* < 0.001 *vs* Control; d*P* < 0.001 *vs* PX+NIC. ABCG2: ATP “binding cassette” G2 drug transporter; O.D.: Optical density; MM: Mecamylamine; MLA: Methyllycaconitine; Lut: Luteolin; nAChRs: Nicotinic acetylcholine receptors; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; PX: Paclitaxel; NIC: Nicotine.



**Figure 9 Possible signal transduction pathways in MDA-MB-231 cells activated by paclitaxel in the absence or presence of nicotine.** NIC: Nicotine; PX: Paclitaxel; nAChR: Nicotinic acetylcholine receptors; ABCG2: ATP “binding cassette” G2 drug transporter; PKC: Protein kinase C; MEK: Mitogen-activated protein kinase kinase; ERK: Extracellular signal-regulated kinases; p38MAPK: p38 Mitogen-activated protein kinases; IKKβ: IκB kinase; IκBα: κB inhibitors.

**Table 1 Effect of the combination of paclitaxel with nicotine on MDA-MB-468 and MCF-7 cell viability**

|  |  |  |
| --- | --- | --- |
| **Treatment** | **MDA-MB-468, cell viability (% of control)** | **MCF-7, cell viability (% of control)** |
| PX | 61.01 ± 3.79 | 65.36 ± 4.86 |
| NIC | 137.79 ± 3.69c | 141.94 ± 4.07c |
| PX+NIC | 79.15 ± 6.94a | 117.99 ± 10.06c |
| PX+NIC+MM | 62.37 ± 4.71 | 69.13 ± 7.22 |

a*P* < 0.05 *vs* control. c*P* < 0.001 *vs* control.