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Relationship between proliferation and percentage displacement in MCF-7 breast cancer cells stimulated with $AlCl_3$ used in women's antiperspirants.

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Abstract*– Breast cancer is the most diagnosed cancer in women worldwide, with more than 2.3 million new cases in 2022. Among the environmental factors potentially involved, aluminum (Al^{3+}) stands out; this metal is present in various cosmetic products and acts as a metalloestrogen by binding to estrogen receptors (ER) and modulating estrogen-dependent expression. Its widespread use as an active ingredient in antiperspirant formulations results in direct and continuous skin exposure in the underarm region, particularly close to the breast tissue. Given the well-established association between estrogen exposure and the development of breast cancer, aluminum could promote abnormal cell proliferation and increase oncogenic risk. However, information on its direct effects on tumor processes remains limited. Therefore, in this study, the effect of $AlCl_3$ on proliferation and migration in MCF-7 breast cancer cells was evaluated using viability (MTT), clonogenic, and cell wound healing assays. The results showed that $AlCl_3$ had an IC_{50} of 12 mM in the MTT assay. At this concentration, colony formation in the clonogenic assay increased significantly ($p < 0.05$), indicating a long-term proliferative effect. However, it did not stimulate cell migration capacity in the wound healing assay. These results suggest that aluminum has a specific proliferative effect on MCF-7 cells without altering their migratory capacity under these experimental conditions, providing relevant evidence for evaluating its involvement in the early stages of breast cancer progression and for its regulation in everyday products, such as women's antiperspirants.

***Keywords*—Breast cancer, MCF-7, Aluminum, Antiperspirants, Cell displacement.**

I. INTRODUCTION

Breast cancer is the most diagnosed cancer in women worldwide, making it a priority public health challenge. According to the International Agency for Research on Cancer (IARC), more than 2.3 million new cases were recorded in 2022 [1], [2], with a mortality rate that makes it the leading cause of cancer death in women worldwide. Projections for 2040 anticipate an increase of more than 35% in incidence and more than 50% in mortality [3]. Breast cancer is characterized by the malignant transformation of breast epithelial cells, which acquire the ability to proliferate abnormally and form a mass known as a tumor. This mass can be detected radiologically as an abnormality in imaging studies (mammography) or manifest clinically as a palpable nodule, which is usually firm and has irregular edges[4]. In advanced stages of the disease, tumor cells acquire invasive properties that allow them to migrate from the primary tumor. These cells manage to infiltrate the lymphatic and blood vessels, using these pathways as conduits to spread to distant sites. When they reach distant organs such as bones, lungs, liver, and brain, they can establish themselves and form new tumor foci, called metastases, which determine a more serious and complex stage of the disease [5].

From a molecular perspective, breast carcinomas are classified into subtypes: the basal subtype (triple negative), lacking estrogen receptors (ER), progesterone receptors (PR), and HER2, accounts for 15–20% of cases and is associated with mutations in TP53/BRCA1, being treated mainly with chemotherapy due to the absence of hormonal targets; luminal A and B subtypes, which express ER/PR, respond to endocrine therapy, and HER2-positive tumors are sensitive to anti-HER2 therapies [6], with the Luminal A subtype being the most common in patients. The increasing incidence of breast cancer has been linked, among other factors, to ongoing exposure to potentially carcinogenic compounds found in everyday consumer goods, including food packaging, personal care products, and medications. Among these, aluminum, a metal frequently used in antiperspirants, which is considered safe, has emerged as a possible agent involved in the pathogenesis of breast cancer [7], [8], [9]. Recent scientific evidence indicates that aluminum may have proliferative effects on breast tumor cells, suggesting a role in disease progression and an adverse impact on the clinical outcome of patients [10]. Considering the background in the scientific community and the consideration of international bodies, such as the Scientific Committee on Consumer Safety [11] is important to establish aluminum as a carcinogen and limit its general use in the population [9]. At the same time, it has been determined that aluminum is a metalloestrogen, meaning it can bind to estrogen receptors (ER) and modulate estrogen-dependent expression. Given the well-established association between estrogen exposure and breast cancer development, aluminum's ability to

mimic estrogen signaling suggests a potential mechanism by which this metal could promote abnormal cell proliferation and increase breast oncogenic risk [12], [13]. However, experimental evidence on its direct effects on key tumor processes remains limited. Therefore, the objective of this study is to examine the effect of aluminum, a component frequently used in women's antiperspirants, on two key processes in breast oncogenesis: cell proliferation and migration (Fig1).

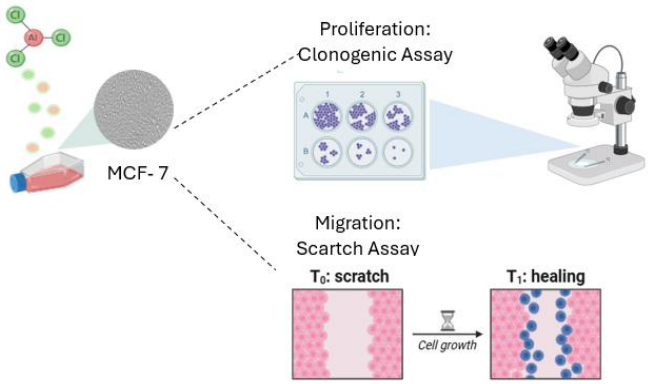

Fig 1. Effect of aluminum on two key processes of proliferation and migration.

II. MATERIALS AND METHODS

A. Cell Culture

The breast cancer cell line, MCF-7, was cultured in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic [14]. The cells were incubated at 37°C, 5% CO_2 until they reached optimal confluence (>80%) for subsequent use.

B. MTT Viability Assay

To evaluate the cytotoxic effect, MCF-7 cells were cultured in 96-well plates ($3x10^4$ cells/mL) and treated with different decreasing concentrations of aluminum chloride (250–0.1 mM) for 24 hours. The IC_{50} analysis was performed using the MTT colorimetric assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)[15]. For this purpose, the wells were treated with MTT solution (final concentration of 0.5 mg/ml) for a period of 3 hours. Subsequently, the plates were washed, and the samples resuspended in DMSO. The reading was performed in the microplate reader at 550 nm [4]. Data processing was performed using GraphPad Prism 8.

C. Clonogenic Assay

MCF-7 cells were cultured in 6-well plates (500 cells/well) for subsequent treatment with $AlCl_3$ (IC_{50}) for a period of 24 hours. Once the treatment time was complete, the cells were incubated, constantly observing the formation of colonies. The experiment ended before colonies consisting of more than 50 cells began to merge, thus ensuring that each colony was clearly distinguishable.

For quantification, Crystal Violet 0.5% was used; colonies of ≥ 50 cells were counted using a stereoscopic microscope, and the data obtained were processed by ImageJ [16], [17], [18].

D. Wound Healing Assay

MCF-7 cells were cultured in 6-well plates ($20x10^4$ cells/ml) with a confluence of >80%. A uniform "wound" opening was made at the bottom of each well using a P200 tip. Subsequently, they were exposed for 24 hours to the following experimental groups: Negative control: cells maintained in DMEM supplemented with 2% SBF; Positive control (standard growth condition): cells in DMEM with 10% SBF; Treated group: cells exposed to the previously determined IC_{50} concentration of $AlCl_3$.

Cell migration was monitored using inverted microscopy at 0, 3, 6, 12, 24, and 48 hours, and the healing area was determined using ImageJ [19].

III. RESULTS

A. IC50 results for MCF-7 treated with $AlCl_3$

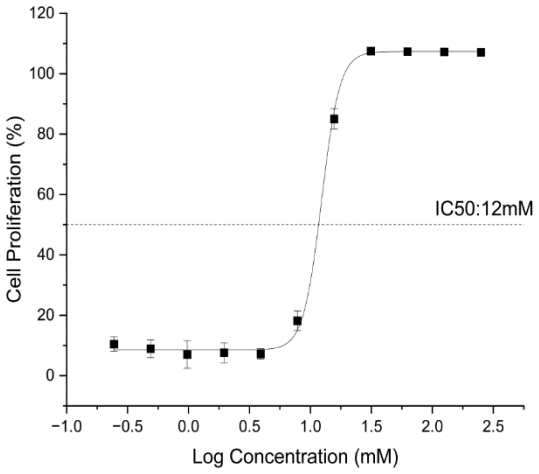

Fig.2. Inhibitory dose-response curve

After exposing MCF-7 cells to different concentrations of $AlCl_3$ for 24 hours, a dose-dependent decrease in cell viability was observed, as assessed by the MTT assay. Analysis of the dose-response curve allowed us to determine a mean inhibitory concentration (IC_{50}) of 12 mM (log 1.07) (Fig 2). At concentrations below 2 mM (log 0.3), the treatment showed no significant cytotoxic effects, maintaining cell viability above 90%. In the range of 3.9 to 7.8 mM (log 0.59 to 0.89), a

progressive reduction in viability was recorded, falling from approximately 90% to around 70%. At concentrations equal to or greater than 15.6 mM (log 1.19), marked inhibition was observed, with viability below 15% at the maximum dose evaluated.

B. Determination of clonogenic capacity

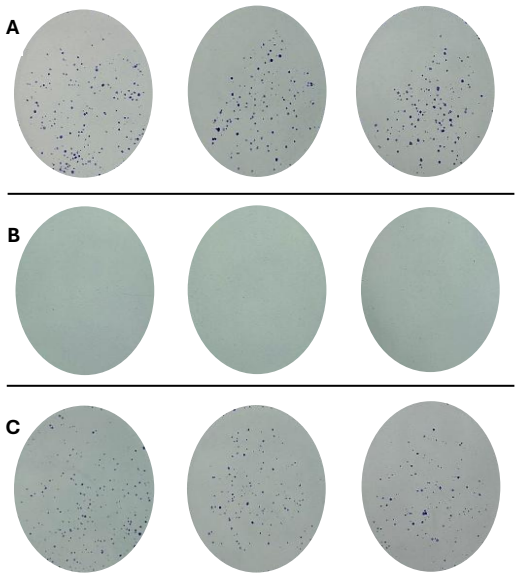

Fig.3. Panel showing the effect of treatment on clonogenic capacity in MCF-7 cells

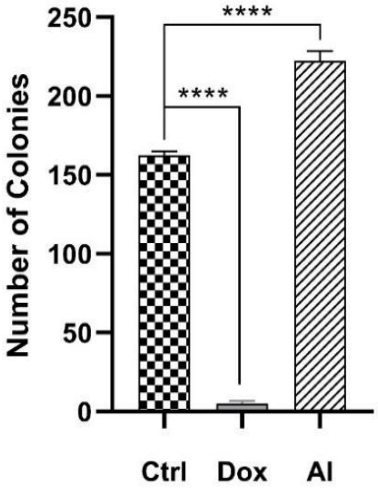

Fig.4. Effect of treatment on clonogenic capacity in MCF-7 cells

The clonogenic assay was used to evaluate the colony-forming capacity of MCF-7 cells after exposure to an $AlCl_3$ concentration equivalent to the IC_{50}. The negative control group (Ctrl) had an average of 162 colonies, while the positive control treated with doxorubicin (Dox) showed inhibition of colony formation, with only 6 colonies detected. In contrast, treatment with $AlCl_3$ induced a significant increase in the number of colonies, with an average of 222 colonies, exceeding both controls. (Fig 3-4).

Statistical analysis shows a statistically significant difference between the experimental groups ($p < 0.05$), demonstrating that $AlCl_3$ stimulates colony formation, validating the proliferative effect of $AlCl_3$ on MCF-7 cells.

C. Determination of displacement capacity

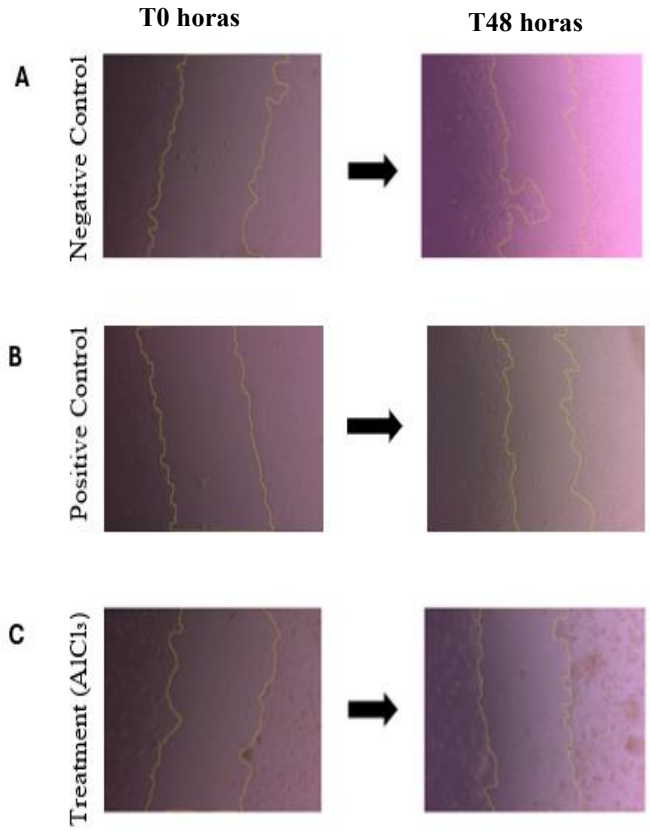

Fig.5. Panel on migration capacity in MCF-7 cells

Representative images of the wound healing assay showing the cell migration front at the initial time point (left) and after the incubation period (right). The cell-free area is outlined in yellow to facilitate identification of the wound edges. A reduction in the uncovered area is observed over time, accompanied by a gradual and irregular advance of the cell front toward the central zone. These images show morphological changes at the edge of the wound consistent with cell migration processes under the experimental conditions evaluated.

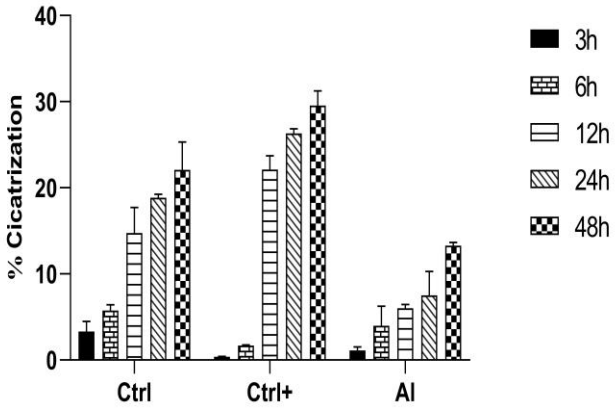

Fig.6. Effect of treatment on migratory capacity in MCF-7 cells

Cell migration was evaluated using a scratch assay on monolayers of MCF-7 cells exposed to an $AlCl_3$ concentration equivalent to the IC50. After 48 hours, the control group showed a 22% closure of the wound area, while treatment with $AlCl_3$ (12 mM) resulted in a 13% closure. Statistically significant differences were observed between the groups ($p < 0.05$). However, the group treated with $AlCl_3$ did not increase cell migration capacity compared to the controls. These results indicate that $AlCl_3$ does not significantly modulate the migration capacity of MCF-7 cells under the experimental conditions evaluated.

IV. DISCUSSION

It has now been established that aluminum has a genotoxic profile, with the ability to induce DNA damage through the generation of oxidative stress. [20]. In the context of breast cancer, this oxidative stress, when moderate, does not act solely as a damaging agent but functions as an intracellular signal that activates key signaling pathways associated with tumor survival and progression, such as MAPK/ERK, PI3K/AKT, and NF-κβ. At the same time, cancer cells respond to moderate levels of reactive oxygen species (ROS) by activating the transcription factor NRF2, which induces the expression of antioxidant and metabolic genes. This metabolic reprogramming, which could also be induced by aluminum, allows the cell to neutralize excess ROS and maintain a redox environment favorable for its proliferation, thus converting oxidative stress into a pro-tumor stimulus [21], [22].

In addition to this mechanism, $AlCl_3$ is considered a "metalloestrogen." [20], [23]. This behavior is particularly relevant in cells that express estrogen receptors, such as the MCF-7 cell line. Aluminum can bind to the ligand-binding domain of the receptor, inducing a conformational change analogous to that caused by estradiol. This binding activates receptor-dependent transcriptional machinery, leading to the expression of estrogen-regulated genes that promote fundamental oncogenic processes such as cell proliferation, survival, and tumor progression [20], [24], [25]. These reports coincide with our clonogenic assay results, which show that stimulation of MCF-7 cells with the IC50 (12 mM) of AlCl3 promotes cell proliferation and survival. (Fig 3-4).

MCF 7 cells did not show significant wound closure in the healing assay after treatment with AlCl3, indicating a clear absence of active cell migration under these experimental conditions. The in vitro wound healing assay primarily evaluates the ability of cells to move and reorganize themselves to fill a space, a process that depends on dynamic cytoskeletal events, substrate interaction, and cell motility-specific signaling pathways, rather than solely on cell proliferation [26]. On the other hand, the MCF 7 cell line, derived from human breast ductal carcinoma, typically shows moderate to low migratory potential in migration assays without the need for exogenous stimuli to promote the activation of migration pathways [27]. Therefore, in the absence of specific signs of migration or associated phenotypic changes, minimal or no migration in the assay is consistent with our results and previous reports on the baseline behavior of MCF-7 [28]

With the above in mind, we report that our results reveal a dissociated response in MCF-7 cells upon exposure to $AlCl_3$. Although a significant increase in cell proliferation was observed, consistent with its metalloestrogenic nature [20], [23] No concomitant increase in the migratory or invasive capacity of these cells was detected. The explanation for this phenomenon may lie in the specific nature of the stimuli generated by $AlCl_3$ and the cellular context in which they act. First, the observed proliferation is consistent with the activation of the estrogen receptor (ERα) by $AlCl_3$, which drives the transcription of proliferative genes through estrogen response elements (ERE)[23]. This is a classic program well characterized in luminal A cells, such as MCF-7, which leads to clonal expansion but not necessarily to epithelial-mesenchymal transition (EMT) or motility. Secondly, it is crucial to consider the concept that proliferation and migration are different cellular processes, often mutually exclusive. [29], [30] where the cell can maintain a proliferative or migratory state, but it should be noted that they are regulated by different and sometimes antagonistic signaling networks.

On the other hand, moderate oxidative stress induced by aluminum may be activating pathways such as PI3K/AKT and MAPK/ERK, which promote survival and cell cycle progression [20], [22], but without reaching the threshold or inducing the specific type of signaling (sustained TGF-β, marked hypoxia signals, specific interactions with the matrix) required to trigger an aggressive migratory phenotype. In this regard, the stimulus provided by $AlCl_3$ may be sufficient to promote growth. However, insufficient to overcome the molecular barriers that suppress motility in this cell subtype. In this case, $AlCl_3$ could be acting as a tumor promoter that

expands the initial cell population, but not as a migration inducer, at least under these conditions.

Darbre *et al* demonstrated that prolonged exposure (weeks) to aluminum salts increased cell migration and invasion in MCF-7 cells, whereas short-term exposure did not [31] suggest that exposure to aluminum may have complex effects depending on time and concentration in cancer cells. This suggests that the time-dependent effects of aluminum may produce more profound alterations in signaling or cumulative epigenetic changes to modify motility. In our case, the exposure time of MCF-7 cells to aluminum salts may not have been sufficient to activate all migration pathways, resulting in proliferation without migration.

V. CONCLUSIONS

MCF-7 cells exposed to aluminum maintained differentiated and stratified tumor behavior. Although they retained significant proliferative capacity and preserved colony formation, they did not show detectable migration in the wound healing assay. This functional decoupling between proliferation and migration suggests that, under these exposure conditions, tumor cells can alternate between predominantly proliferative or migratory states, rather than executing both processes simultaneously.

Overall, the results shown underscore the importance of evaluating multiple cellular parameters independently and complementarily to interpret the effects of compounds such as aluminum on tumor progression.

This study provides experimental evidence that sustained proliferation can occur without concomitant migration under brief stimuli. Furthermore, these results highlight the need for future studies exploring the effects of longer exposures to aluminum chloride in breast cancer models, as well as research aimed at elucidating the molecular mechanisms and signaling pathways underlying this dissociated cellular behavior.

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