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Presence of Lamin A is Required for GATA3 and ERα Downregulation by Histone Deacetylase Inhibitor in Breast Cancer Cells

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Abstract **Background**: Breast cancer treatment is challenging due to the inconsistence in tumour biomarker expression including progesterone receptor (PR), estrogen receptor-α (ERα) and GATA3 transcription factor. GATA3 has role in epithelial cell differentiation along with nuclear envelope protein lamin A. The breast cancer cell line MCF7 expresses ERα, abnormal GATA3 isoforms, low PR but lacks lamin A. MCF7 cells are resistant to tamoxifen targeting ERα and more anticancer drugs are being studied to kill them. One of the mechanisms surrounding breast cancer initiation is histone deacetylation. Our objective is to investigate the effect of histone deacetylase inhibitor (HDACI) on PR, ERα and GATA3 expression along with the induction of apoptosis in MCF7 cells forced expressing lamin A. Subsequently, they are also explored as biomarkers for breast cancer prognostic and indicators for targeted breast cancer therapy. **Methods:** The HDACI used here is Suberoyl-Bis-Hydroxamic Acid (SBHA). Western blot was used to analyze the expression of PR, ERα, and GATA3 in MCF7 control transfected with histone H2B-GFP (MCF7-H2B-GFP) and in MCF7 transfected with lamin A-RFP (MCF7-LA-RFP). The analyses were carried out before and after treatment with DMSO (mock) or SBHA (1 or 2 µM) for 12h. The *in vivo* expression of the PR, ERα and GATA3 were also explored in 36 archived cell lysates derived from breast cancer micro-biopsies. **Results:** MCF7-H2B-GFP treated with SBHA increased PR, ERα while MCF7-LA-RFP treated with SBHA reduced ERα and GATA3 but not PR. Biomarkers analysis in ductal carcinoma micro-biopsies derived samples showed that 30% had lost lamin A while 64% expressed PR, ERα and GATA3. **Conclusion**: In presence of lamin A, SBHA downregulated cancer initiators GATA3 and ERα while inducing cell death. These biomarkers could be useful molecular tools prior to initiating targeted breast cancer therapy.

Keywords: *Suberoyl-Bis-Hydroxamic Acid, GATA3, ERα, targeted breast cancer therapy*

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1. Introduction

The transcription factor GATA3 is involved in breast epithelial cell differentiation [1]. In breast cancer cell lines, several patterns of GATA3 was reported including gene mutations in MCF7 cell line or loss of expression [1,2]. MCF7 cell line lacks the expression of cell differentiation biomarkers lamin A/C and expresses mutated forms of GATA3 [4,5]. The loss of lamin A function was reported to be one of molecular mechanisms initiating chromosomal instability prior to carcinogenesis [4]. The loss of GATA3 function was associated to invasive tumour and poor prognostic [5]. GATA3 and estrogen receptor alpha (ERα) are involved in a positive feed-back regulation loop [6]. GATA-3 is required for normal mammary gland development and its expression is highly correlated with ERα in human breast tumours [6]. Activated progesterone receptor (PR) reduces GATA3 expression at the transcriptional and post-translational levels in breast cancer cells [1]. PR reduces GATA3 through dual regulation mechanism including epigenetic methylation at GATA3 promotor and protein phosphorylation followed by proteasomes mediated degradation [1]. Most importantly, progestin-induced *in vitro* and *in vivo* breast cancer cell growth required downregulation of GATA3 [1]. MCF7 expresses isoforms of GATA3 and may be resistance to tamoxifen treatment all of which directed to the necessity to unveil other pathways and potent anti-cancer therapy [5,6,7,8]. As such, histone deacetylase inhibitor (HDACI) drugs are now considered for breast cancer therapy [9,10].

Most HDACI drugs can overcome epigenetic modifications encountered in some breast cancers [9,10]. The HDACI Suberoyl-Bis-Hydroxamic-acid (SBHA) was reported to be a potent anti-breast cancer drug that uses the activation of the p53/p21 pathways to induce apoptosis in MCF7 cell line [9,10]. Extensive investigation showed that the upregulation of p53, p21 and Bax was involved in SBHA-induced apoptosis in MCF-7 cells. [9,10]. To the best of our acknowledges, there was no investigation on the effect of SBHA on cell differentiation marker GATA3, its transcriptional regulator PR and its transcriptional target ER in presence or absence of lamin A. Our recent investigation focused on this aspect to contribute to the understanding of the mechanism underlying MCF7 cell sensitivity to SBHA. The pattern of PR, ERα and GATA3 are variable in breast cancer cells and should be taken into consideration when establishing breast cancer therapy. The evaluation of lamin A, PR, ERα and GATA3 in breast cancer micro-biopsies could also be useful to delineate potent breast cancer therapies. Our objective is to investigate the effects of histone deacetylase inhibitor (HDACI) on PR, ERα and GATA3 expression in MCF7 cells forced expressing lamin A. Subsequently, they are also explored as biomarkers for breast cancer prognosis and therapy.

2. Methods

2.1. Materials and Reagents

Tris-Base, glycine, sodium dodecyl sulfate, Sodium chloride (NaCl), potassium chloride (KCl), Tween-20, protease inhibitor phenyl-methyl-sulfonyl fluoride (PMSF), 2-mercaptoethanol, methanol, glycerol, 1,4-Dithiothreitol (DTT), sodium fluoride (NaF), sodium azide (NaN3), Tris-Hydrochloride (tris-HCl) and sodium dodecyl sulfate (SDS, NaC12H25SO4), Dimethyl Sulfoxyde (DMSO) and n-propyl-gallate were purchased from Sigma-Aldrich (USA). Bis-acrylamide, nitrocellulose membrane, protein ladders and peroxidase conjugated secondary antibodies anti-rabbit and anti-mouse were purchased from Bio-Rad Inc (USA). Suberoyl-Bis-Hydroxamic-Acid (SBHA) powder was from BIOMOL (USA). The primary antibodies against lamin A/C, GATA3, progesterone receptor (PR), estrogen receptor alpha (ERα) and Caspase 3 were from Santa Cruz Biotechnology (CA, USA). Primary antibody against ß-actin (mouse IgG) was from transduction lab BD Bioscience (USA). The secondary antibodies against mouse or rabbit were from Bio-Rad Inc (USA). The protein detection reagent "Super Signal West Dura Extended Duration Substrate" made by PIERCE was from Thermo Scientific (Rockford, IL USA). X-ray films were from Kodak (USA). Cell culture dishes, cell culture media (DMEM and Opti-MEM), antibiotics penicillin/streptomycin and fetal bovine serum-albumin (FBS) were from Fisher Scientific (USA). Breast cancer cell lines (MCF7, T47D) as well as recombinant plasmids with histone H2B gene fused to green fluorescent protein (PCDNA3-H2BGFP), lamin A gene fused to red fluorescent protein (PCDNA3-LA-RFP), lamin C gene fused to green fluorescent protein (PCDNA3-LC-GFP) were gifts from Pr. Xiang-xi Xu's Lab [4]. All breast cancer cell lysates derived from micro-biopsies were obtained from National University Hospital (CNHU-HKZ) in BENIN.

2.2. Cell Culture

Breast cancer cell lines were seeded in 6 well cell culture dishes and incubated in DMEM medium containing 10% FBS and antibiotic. For drug experiment cell culture medium was supplemented with DMSO (mock) or with histone deacetylase inhibitor SBHA (1 or 2µM). DMEM medium containing SBHA was prepared freshly from a stock solution of SBHA in DMSO (100 mg/ml). Cancer cells were exposed to DMEM medium with SBHA or DMEM medium with DMSO for 12 h. For protein analysis with western blot technic (immunoblotting), cells were scraped and collected in 15 ml falcon tubes, rinsed with PBS and lysed with RIPA buffer [4].

2.3. Cell Transfection and Selection

The procedure for cell transfection and selection follows the one previously described [4,9]. In brief MCF7 cells were seeded in 6 wells cell culture dish and transfected with PCDNA3-H2BGFP, PCDNA3-LARFP or PCDNA3-LCGFP in 1 ml serum free medium for 8 h. Then, 1 ml of DMEM containing 20% FBS was added and cultured for 24 h. To achieve stable transfection, cells were cultured in DMEM medium containing antibiotics (Penicillin/Streptomycin 1x, neomycin "G418" 1nM) and 10% FBS. Cells were cultured one more week and fluorescent cells were sorted by flow cytometer as previously described [4,11].

2.4. Immunofluorescence and Metaphase Count

MCF7 cells stably expressing histone H2B-GFP, lamin A-RFP or lamin C-GFP were cultured for 3 days inside 6 well dishes in dye free DMEM medium and visualized directly under Zeiss fluorescence microscope linked to Zeiss AxioCam camera. Pictures were taken with AxioVision Rel.4.8 software. To count metaphase, adherent cells expressing H2BGFP, lamin A-RFP or lamin C-GFP were cultured for 24 h on top of glass cover slips placed inside 6 well dishes. To synchronize cells, they were serum for 12 h. Cell mitosis was induced by culturing them in DMEM medium containing 10% FBS and antibiotics for 4h. At time point of 1 h, 2 h, 3 h and 4h, cells were washed twice with PBS at room temperature, fixed with 4 % paraformaldehyde for 15 min and permeabilized with 0.5 % Triton X-100 for 5 min. Cells were washed three times with PBS, blocked with 3% BSA in PBS containing 0.1 % Tween-20 for 30 min before incubation for 5 min in Hoechst 33342 solution to stain nucleus and chromosomes. Cells were washed again three times, mounted in anti-fade reagent made with 100 mM of n-propyl-gallate (pH 7.4), 90 % glycerol in PBS and sealed with nail polish. Cell with metaphases and whole nucleus were counted under immunofluorescence microscope Zeiss with 100× oil objective lens. Image acquisition was done using AxionVision 4.8 software [4,11]. Our study will focus on lamin A-RFP as it is involved in cell cycle regulation [4].

2.5. Cell Lysate Processing

- **Cell culture lysate**: cell culture media were discarded, and cells were washed with PBS. Cell pellet was lysed on ice for 30 min with 200 µl cell lysate buffer A [50 mM Tris-HCl (pH 7.9), 150 mM NaCl, 0.1 mM EDTA, 1 % NP-40, 0.5 mM DTT, 0.5 mM PMSF, 30 mM NaF and 0.5 % protease inhibitor cocktail]. Protein denaturation was achieved with 50 µl (4 x stock) SDS sample buffer B followed by boiling at 95°C for 5 min and store in freezer (-20° C) until needed for analysis [4,11,12].

- **Micro-biopsies lysate**: breast cancer micro-biopsies were performed in the National University Hospital (CNHU-HKZ) of Benin following the procedure for pilot targeted cancer-therapy supervised by oncological surgeon Dr. Gnangnon F and visceral surgeon Pr. Olory-Togbé JL. Written signed informed consent was obtained from the participants prior to the procedure. Our research procedure followed the guide lines of Helsinki's declaration. The research ethics committee of the Institute of Biomedical Sciences and Applications (CER-ISBA) approved our procedure [12]. Samples from breast micro-biopsies were crushed and lysed by incubation with 200 µl of ice cold cell lysis buffer A and kept on ice for 30 min with vigorous agitation every 5 min. A volume of 200 µl SDS sample buffer (2 x) was added to cell lysate before boiling at 95°C for 5 min and stored in freezer (-20°C) until needed for analysis [11,12].

2.6. Cell Processing for Western Blot

Before western blotting, protein samples were boiled again and loaded on 7-12 % SDS-polyacrylamide gels. Electrophoresis was run at 100 volts for 2 h in tris-glycine buffer, followed by a transfer on nitrocellulose membranes with transfer buffer containing Tris-glycine and 20 % methanol. Membranes were blocked with 5 % milk in 1X Tris-buffered-saline (TBS) containing 0.1 % Tween-20 (TBST) for 30 min at room temperature before incubation in primary antibody rabbit-anti-lamin A/C or mouse anti β-actin at room temperature. The membranes were washed 4 times for 10 min with TBST and incubated with HRP-conjugated secondary antibody anti-rabbit or anti-mouse for 1 h followed by washes with TBST. Then membranes were incubated for 3 min in Super Signal West Dura Extended Duration Substrate before exposure to premium blue x-ray films (Phenix, research products, USA)). X-ray film processor was used to reveal proteins as dark bands [11,12].

2.7. Micro-biopsy Lysate Data Analysis

To analyzes the non-parametric data and present them in diagramme, normal protein expression was assigned with the value 2, weak protein expression value 1 and lack of protein expression value 0. The difference in expression pattern between GATA3 and ERα expression; GATA3 and PR expression; or between ERα and PR was analyzed with F-test. The differences were considered statistically significant when $p < 0.05$.

2.8 Ethical Approval and Consent to Participate

The Institute of Biomedical Sciences and Applications (CER-ISBA) approves this pilot study. Procedures followed in this study were in accordance of our institute ethical guidelines data obtained could not be traced to individuals. Written and signed Informed consent was obtained from the patient before sample collection and analyses.

3. Results

3.1. Deficiency of Lamin A/C and Caspase 3 in MCF7 Cell Line

Lamin A/C and caspase 3 known respectively as cell differentiation marker and apoptosis executioner, are normally expressed in normal breast epithelial cells (NBE) and in immortalized breast epithelial cell line (MCF10). Lamin A/C and caspase3 are absent in MCF7 unlike to breast cancer cell T47D (Figure 1).

3.2. Transfection of Lamin A and Lamin C in MCF7 Slows down Cell Division Rate but do not Induce Apoptosis

To verify the effect of lamin A and lamin C on cell division, MCF-7 cells were stably transfected with recombinant PCDNA3-H2B-GFP, PCDNA3-LA-RFP or PCDNA3-LC-GFP. Cells were cultured for 12 h in serum free media for synchronization and cell division was activated with media containing 20% serum. Cells were washed and fixed for 1 h after stimulation of cell division. DNA was stained with DAPI and number of metaphases were counted. Cell division rate was evaluated by percent of metaphases per hour. We noticed that in presence of lamin A-RFP or lamin C-GFP cell mitosis was significantly reduced (4.4 and 3.6%) compared to MCF7 control transfected with histone H2B-GFP (22%) as displayed by histogram in Figure 2. Transfection of lamin A-RFP or lamin C-GFP reduces significantly the number of cell undergoing mitosis (Figure 2).

MCF7 transfected with Histone H2B-GFP (control) and MCF7 expressing exogenous lamin A-RFP (MCF7-LARFP) were used for this study (Figure 3). Exogenous lamin A slows down cell growth (Figure 2) but did not induce apoptosis (Figure 3).

3.3. Histone Deacetylase Inhibitor (SBHA) Induces Apoptosis in MCF7

To evaluate the influence of lamin A-RFP on breast cancer cell growth regulation after treatment with SBHA, we treated them with anti-cancer drug SBHA known to be a potent inhibitor of histone deacetylase. Our results showed that SBHA induced MCF7 cell apoptosis in dose dependent manner (Figure 4). Apoptosis was not observed in MCF7 control treated with DMSO (A) but was observed in MCF7 treated with 0.5 µM of SBHA (B); 1 µM of SBHA (C); and 2 µM of SBHA(D). Cell death is more significant in MCF7 treated with 2 µM of SBHA (D) than 1 µM of SBHA (C) or 0.5 µM of SBHA (B) compared to the control (A) as shown by bright field imaging (Figure 4). All experiments were carried out three times.

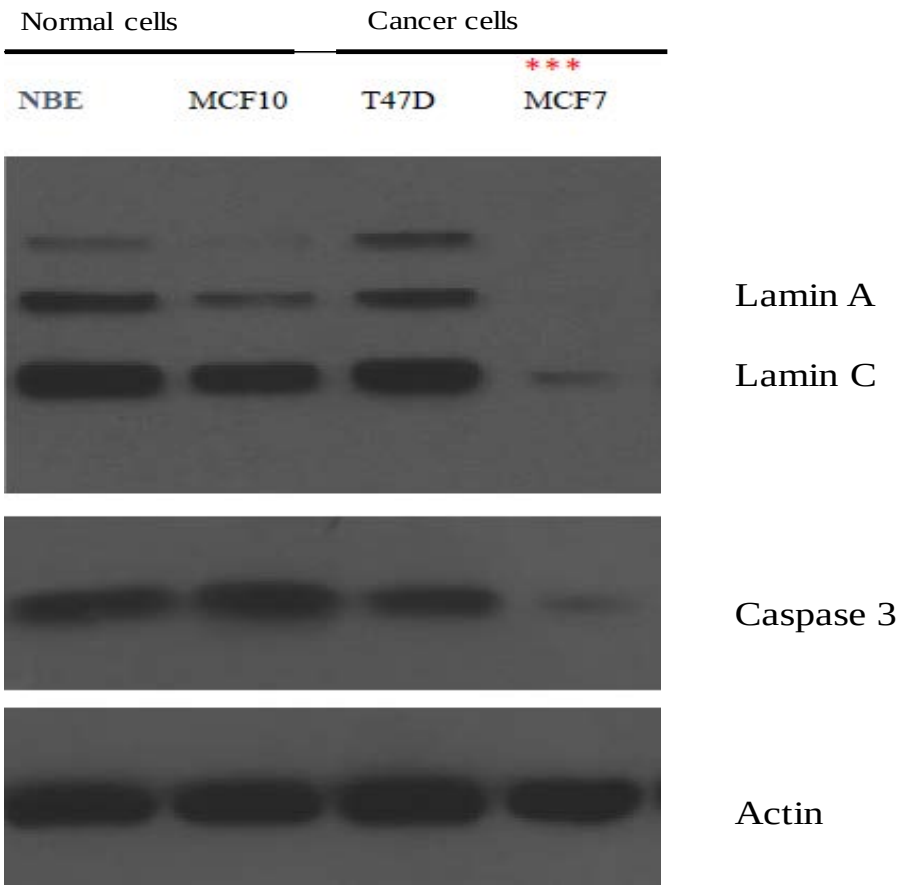

Figure 1. Expression of lamin A/C and caspase 3 are observed in normal breast epithelial cells (NBE) and in immortalized breast epithelial cell line (MCF10). Breast cancer cells T47D expresses cell differentiation biomarker lamin A/C and apoptosis marker caspase3. In contrast, MCF7 do no express lamin A/C nor caspase3

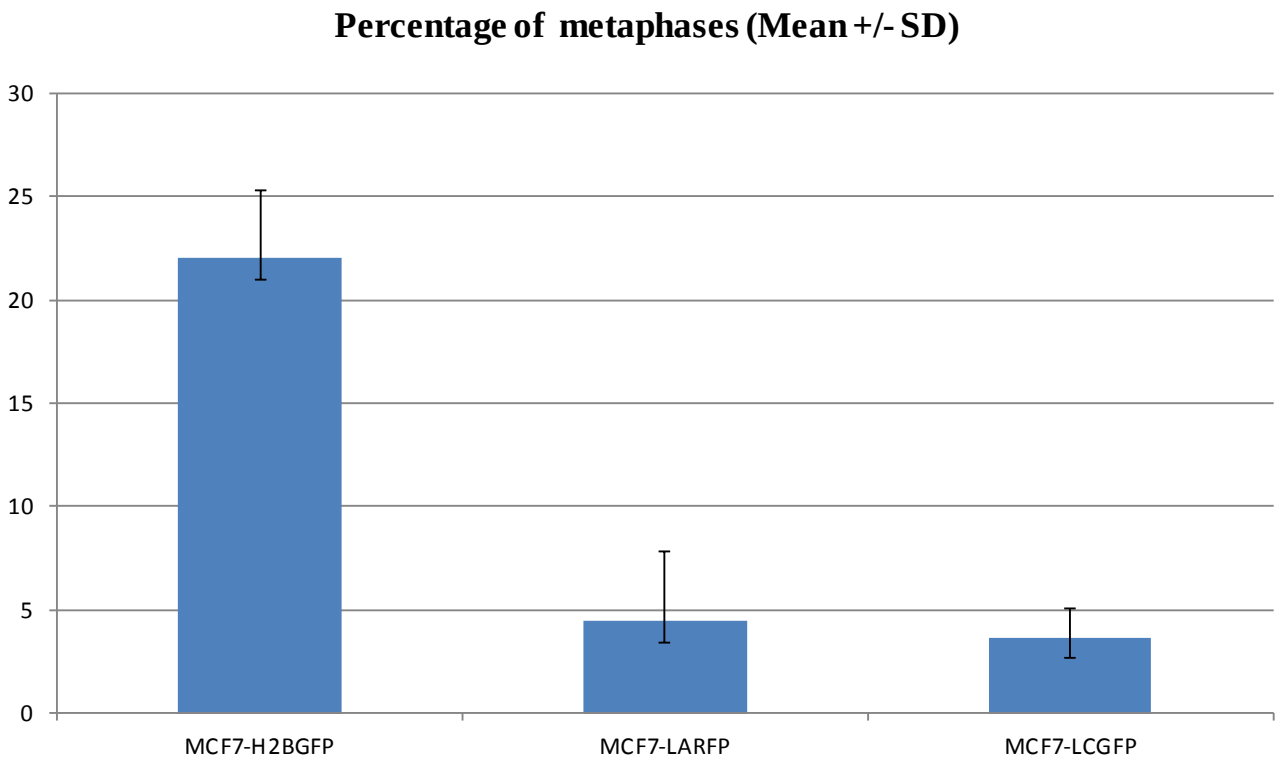

Figure 2. Percentage of metaphases in MCF7 cells stably transfected with histone H2B-GFP, Lamin A-RFP, or Lamin C-GFP. MCF7 proliferation is significantly reduced in presence of lamin A-RFP (4.4%) or lamin C-GFP (3.6%) in contrast to the control transfected with PCDNA3-H2B-GFP (22%)

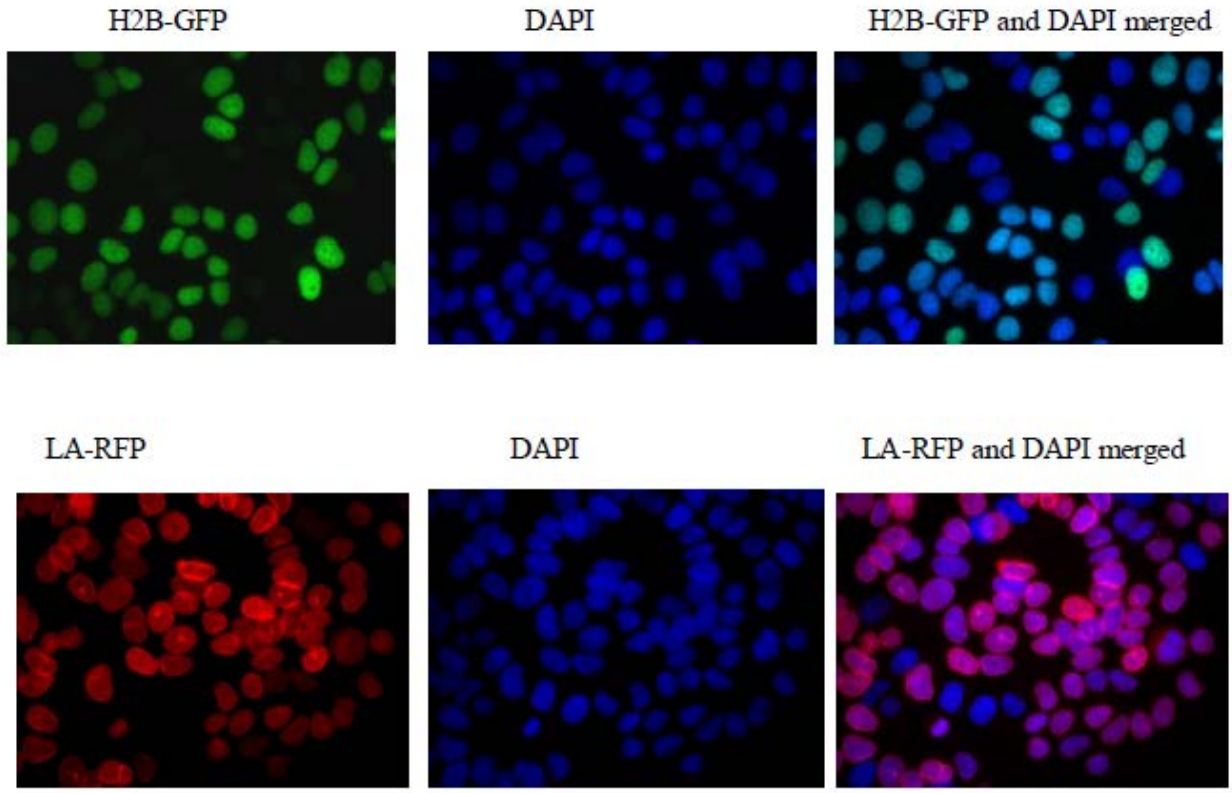

Figure 3. Fluorescence microscopy images showing MCF7 stably transfected with H2B-GFP and lamin A-RFP plasmids. Exogenous lamin A-RFP was well localized around nuclei. No apoptotic cells were observed

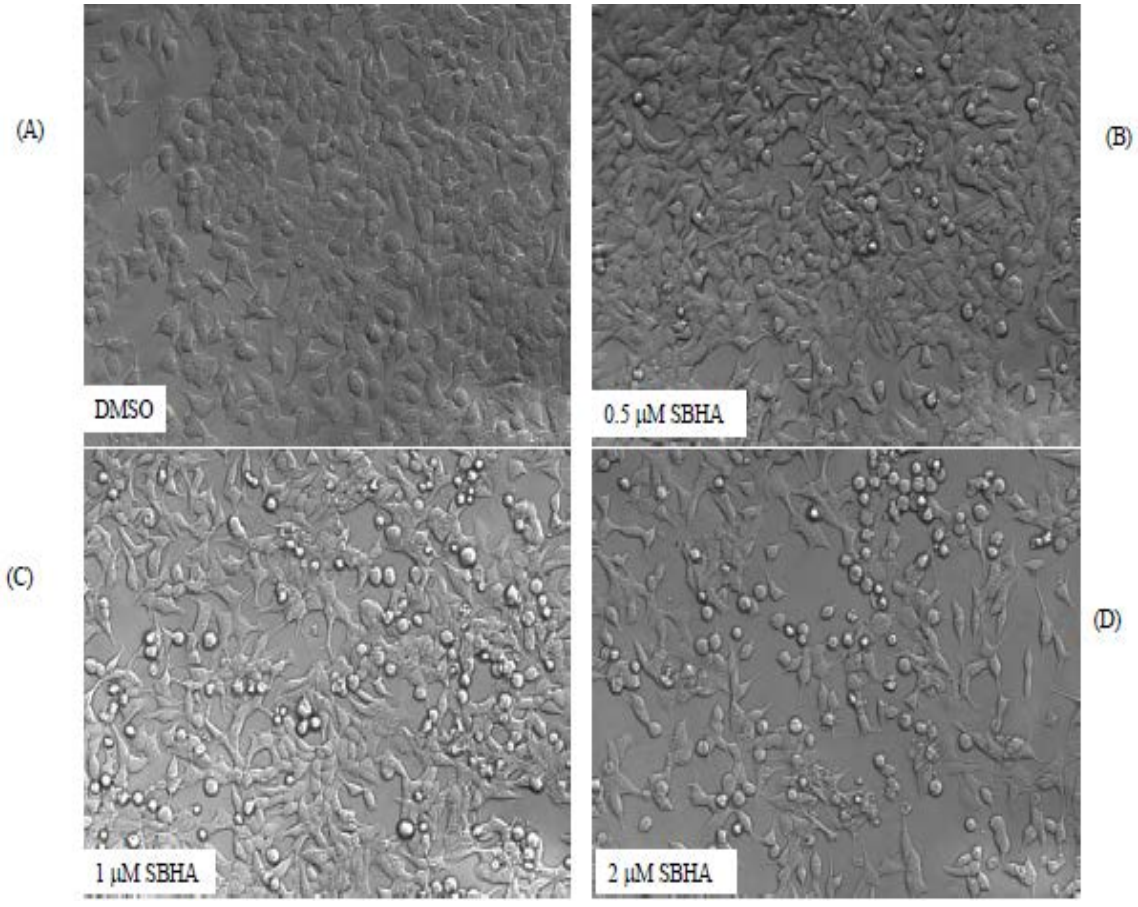

Figure 4. Microscopy images showing the effect of Histone deacetylase inhibitor SBHA on MCF7 cells. This effect is dose dependent: (A) MCF7 control treated with DMSO (mock) for 12h compared to MCF7 treated for 12 h with (B) 0.5 µM SBHA; (C) 1 µM SBHA and (D) 2 µM SBHA. Apoptosis (rounded cells) is induced in a dose dependent manner

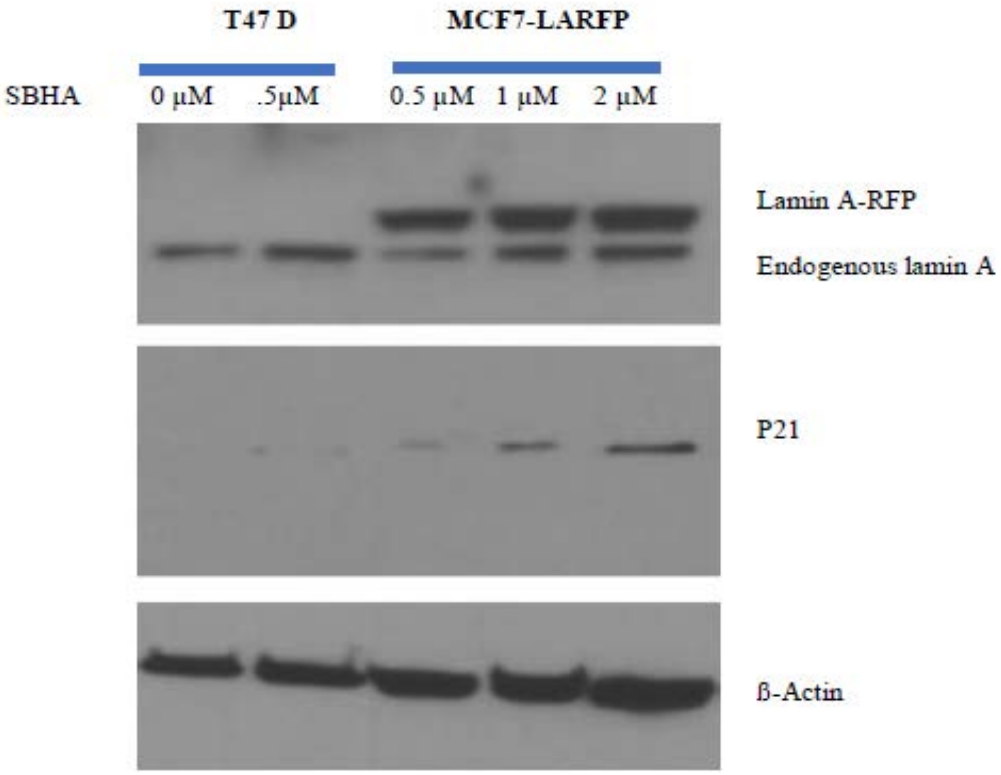

Figure 5. SBHA induces the expression of endogenous lamin A in T47D and in MCF7 transfected with-LA-RFP. SBHA induces also the expression of P21 in MCF7 transfected with lamin A-RFP, in a dose dependent manner

3.4. Treatment of Breast Cancer Cells with SBHA Restores Endogenous Lamin A Expression in MCF7

To verify the potency of SBHA to induce endogenous lamin A and P21 expression, we treated MCF7-LARFP with DMSO and different concentrations of 0.5µM - 2µM SBHA in DMSO for 12h; T47D is another breast cancer cell line treated as positive control for endogenous lamin A. DMSO did not induce lamin A but a concentration of 0.5µM SBHA was sufficient to induce endogenous lamin A in T47D and MCF7-LARFP. The induction of endogenous lamin A increased with SBHA concentration; an example of dose dependent induction of endogenous lamin A was shown for MCF7-LARFP cell lysate in Figure 5. Similarly, the induction of cell cycle regulation protein P21 in MCF7-LARFP was also dose dependent; ß -actin was used as loading control (Figure 5).

3.5. In presence of Lamin A-RFP Treatment with SBHA Reduces GATA3 and ERα on the Pathway to induce MCF7 Cell Death

MCF7 expressed low level of PR, noticeable level of ERα and isoforms of wild type and mutated GATA3 ($GATA3^{w/m}$); β-actin was used as loading control as shown by western blot (Figure 6). MCF7 transfected with histone H2B-GFP plasmid (MCF-H2B-GFP) and treated with DMSO did not display effect on PR, ERα or $GATA3^{w/m}$ expression. MCF-H2B-GFP treated with SBHA induced PR, ERα and $GATA3^{w/m}$ compared to MCF-H2B-GFP treated with DMSO as control (Figure 6). In contrast, MCF7 transfected with lamin A (MCF-LA-RFP) and treated with DMSO showed higher expression of PR and GATA3 but not ERα. Treatment of MCF-LA-RFP with SBHA (1µM or 2 µM) for 12 h leads to minor reduction of PR, and noticeable reduction of ERα and $GATA3^{w/m}$. In presence of LA-RFP, SBHA contributes significantly to the reduction of ERα and $GATA3^{w/m}$ involved in breast cell proliferation and carcinogenesis. SBHA alone did not reduce ERα (Figure 6).

3.6. Assessment of GATA3, ERα and PR in Breast Cancer Micro-biopsies and Contribution to Targeted Therapy

Heterogenous expression of GATA3, ERα and PR was observed in breast cancer micro-biopsies **(n = 36)** and their assessment could contribute to targeted therapy. Lamin A was absent in 30% of breast ductal carcinoma microbiopsies. The evaluation of PR, ERα and GATA3 expression pattern reveals that: (a) PR, ERα and GATA3 were present in 23/36 (64%) of ductal carcinomas; (b) PR, ERα but not GATA3 were observed in 4/36 (11%) of ductal carcinomas, (c) PR, GATA3 but not ERα were observed in 6/36 (16%) of ductal carcinomas; (d) PR but not ERα nor GATA3 was observed in 1/36 (3%) of ductal carcinomas; (e) GATA3 but not PR or ERα was observed in 1/36 (3%) of ductal carcinomas; (f) PR, ERα and GATA3 were absent in 1/36 (3%) of ductal carcinomas. The diagram showing the expression pattern of PR, ERα and GATA3 in breast cancer micro biopsies is presented in Figure 7 and demonstrated that PR, ERα and GATA3 patterns varied among patients. Most of cases were PR, ERα and GATA3 positive (64%) followed by PR positive, ERα and GATA3 negative (16%).

Overall, 29/36 (80%) of breast cancer patients expressed isoforms of GATA3 and PR; 23/36 (64%) of

breast cancer patients expressed isoforms of GATA3 and ERα. Comprehensive analysis pointed out that 23/36 (64%) of breast cancer patients expressed isoforms of GATA3, ERα and PR while 9/39 (25%) of patients do not express ERα. No significant differences were observed between the expression of GATA3 and ERα ($p = 0.94$); GATA3 and PR ($p = 0.18$); or between ER and PR ($p = 0.16$). In 64% of cases there is correlation between GATA3 and ERα with heterogenous expression of wild type and mutated isoforms of GATA3 (GATA3 w and GATA3m).

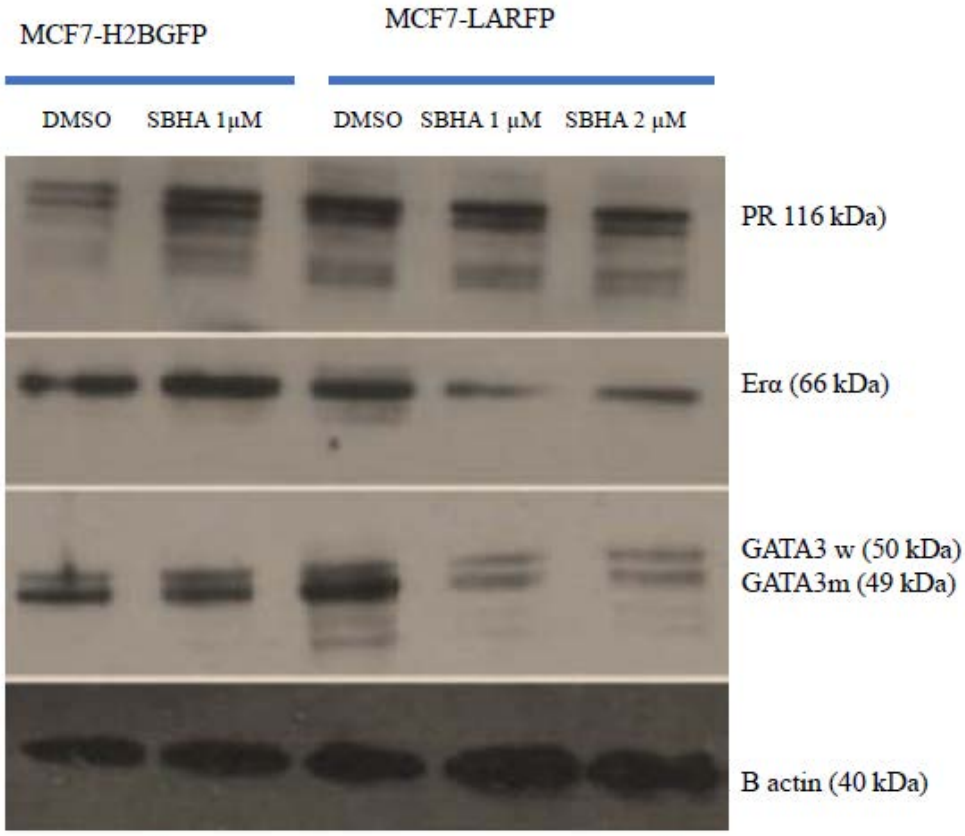

Figure 6. Western blot showing the expression level of progesterone receptor (PR), estrogen receptor alpha (ERα) and GATA3 in MCF7-H2B-GFP and MCF7-LA-RFP treated with DMSO (mock control) and SBHA (anti-cancer drug inhibitor of histone deacetylase). Forced expression of LA-RFP enhanced the reduction of ERα and GATA3 in MCF7 cell line. β-actin was used as loading control. GATA3 w (wild type), GATA3m (mutated).

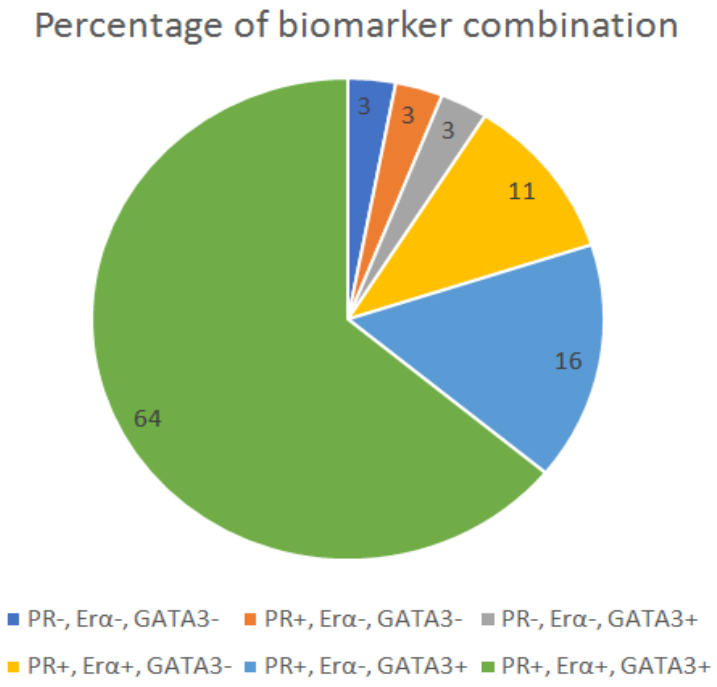

Figure 7. Diagram showing expression pattern of PR, ERα and GATA3 patterns in breast cancer micro biopsies. Most of cases are PR, ERα and GATA3 positive (64%) followed by PR positive, ERα and GATA3 negative (16%)

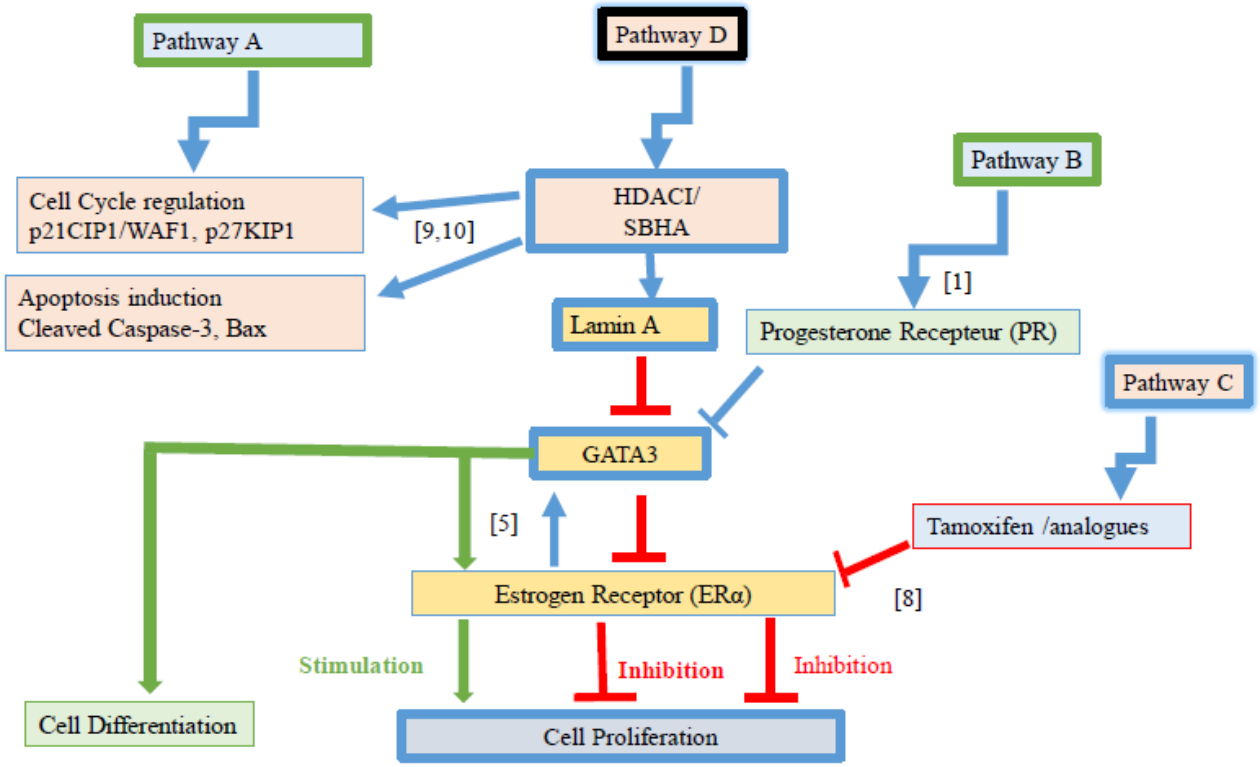

Figure 8. Representation of multiple pathways used by anti-cancer drugs such as tamoxifen and analogues, histone deacetylase inhibitors (HDACI/SBHA) to regulate cell proliferation, induce apoptosis or cell differentiation. Pathways A, B and C were documented [1,8,9,10]. Our study suggested that SBHA may use pathway D in presence of lamin A to inhibit breast cancer cell growth.

3.7. Multiple Pathways are Involved in Breast Cancer Initiation and Therapies

Breast cancer initiation could be associated to polymorphism or deficiency of GATA3, overexpression of ERα or PR. Targeted breast cancer therapy should be used to achieve proper cell growth regulation, apoptosis, or differentiation to overcome breast cancer (Figure 8). We displayed multiple pathways used by anti-cancer drugs such as tamoxifen and analogues, histone deacetylase inhibitors (HDACI/SBHA) to regulate cell proliferation, induce apoptosis or cell differentiation. Pathways A, B and C were documented [1,8,9,10] while further works are needed to unveil pathway D associated with the down regulation of GATA3 in presence of lamin A and SBHA (Figure 8). GATA3 has dual effects including stimulation of cell differentiation and inhibition of cell proliferation through inhibition of estrogen receptor (ERα). Our study suggested that SBHA may use pathway D in presence of lamin A to inhibit GATA3, ERα and subsequently breast cancer cell proliferation.

4. Discussion

The molecular mechanisms initiators of cell proliferation and breast cancers are diverse and complex. GATA3 variants may contribute to tumorigenesis in ERα-positive breast tumors and to differential drug sensitivity [2,3]. GATA-3 stimulates ERα transcription through binding to two cis-regulatory elements and recruiting RNA polymerase II at ERα promoters [6]. MCF7 cell line lacks the expression of cell differentiation biomarkers lamin A/C and harbors a polymorphism of GATA3 transcription factor gene, all of which may favor molecular pathway leading to resistance of this cell line to ERα inhibitors including tamoxifen [4,5,8]. According to our data, GATA3 is a target of histone deacetylase inhibitor SBHA through PR pathway in MCF7 breast cancer cell line forced expressing lamin A-RFP. This was not observed in the control MCF7 forced expressing histone H2B-GFP. The molecular mechanism used by SBHA to increase PR, downregulate ERα and GATA3 expression seems to require the presence of lamin A in the pathway to inhibit cell growth.

Our preliminary data suggested that GATA3 may have dual effects in breast cancer cells including stimulation of cell differentiation and inhibition of cell proliferation through stimulation or inhibition of ERα. Thus, the observation that GATA3 downregulation is required for PR induces breast cancer cell growth reported previously is no longer applicable in presence of lamin A [1,7,8]. Recent study showed that truncated form of GATA3 has role in breast carcinogenesis and resistance to some anticancer therapies [3]. Low expression of GATA3 was observed in breast invasive carcinomas with poor clinical outcome [5]. Nevertheless, the status of GATA3 during cancer treatment was not thoroughly studied.

Our study shows that the abnormal GATA3 in MCF7 cell line could be reduced by SBHA in presence of exogenous lamin A. Treatment of MCF7-H2BGFP with SBHA could restore lamin A and increase PR along with GATA3. Surprisingly, in presence of lamin A-RFP, SBHA increased PR expression while downregulating GATA3 and ERα expression. This observation is in accordance with the study reporting that activated PR reduces GATA3 expression at the transcriptional and post-translational levels in breast cancer cells. The reduction is

achieved through dual regulation mechanism including epigenetic methylation at GATA3 promotor, protein phosphorylation and subsequently degradation mediated by proteasomes [1]. Although some breast cancer cells may express abnormal isoforms of lamin A, in presence or absence of lamin A cancer cell death was observed with SBHA. Thus, breast cancer cells lacking lamin A used GATA3 independent pathway to respond to SBHA-induced cancer cell death signal. In contrast, breast cancer cells expressing lamin A reduced GATA3 and ERα in the pathway to induction of cell death.

Overall, breast cancer cells sensitivity to SBHA could be monitored by the expression of lamin A, PR, GATA3, and ERα. GATA-3 may be required for estradiol stimulation of ERα-positive breast cancer cell proliferation [6]. SBHA is a good anti-cancer drug to inhibit cell proliferation through inhibition of both GATA3 and ERα in presence of lamin A.

Beside gene mutations, all molecular mechanisms linked to epigenetic modifications could be restored with HDACI [8,10,13]. Further analysis with ChIP-seq will be needed to reveal proteins that interact with GATA3 and lamin A in the presence of SBHA [14,15]. Nevertheless, SBHA treatment of breast cancer cells lacking lamin A and GATA3 led to the induction of lamin-A and GATA3 all of which are biomarkers for good prognosis. In cancer cells expressing lamin A and GATA3, treatment with SBHA could lead to a reduction of GATA3 and ERα as sign of excellent response to anti-cancer therapy. The use of cell lysates from breast micro-biopsy samples to analyze the expression pattern of PR, ERα and GATA3 will help to establish targeted anti-cancer therapy and evaluate prognosis. In consideration to the breast cancer micro-biopsies, 64% of women with ductal carcinoma cells expressing PR, ERα and GATA3 will be good candidates for SBHA treatment. The 25% of patient samples without ERα could also benefit from SBHA treatment in presence of lamin A and GATA3.

In this study, all ductal carcinoma samples expressing GATA3 displayed wild type and mutated isoforms as observed in MCF7 cell lines by western blot. The distinction between GATA3 wild type and mutated isoforms is not possible with immunohistochemistry. Cancer-derived mutation in GATA3 deregulated physiologic protein turnover, stabilized GATA3 binding across the genome and modulated the response of breast cancer cells to estrogen signaling [15]. Our present study showed that SBHA can disrupt the effect of mutated GATA3 on ERα induced breast cancer cell proliferation.

5. Conclusion

Overall, treatment of breast cancer cell lines with SBHA restored endogenous lamin A expression which has no effect on GATA3 and ERα compared to wild type exogenous lamin A. Among the variety of drugs approved for breast cancer treatments, *in vitro* testing should be done on micro-biopsy cell lysates of each patient to delineate potent therapy regarding cytotoxicity, cell cycle regulation, cell apoptosis and restoration of cell differentiation biomarkers.

List of abbreviations

ERα, estrogen receptor alpha; GATA3, transcription factor; GFP, green fluorescent protein; HDAC, histone deacetylase inhibitor; HRP, Horseradish Peroxidase; KCl, potassium chloride; RFP, red fluorescent protein; NaCl, Sodium chloride; NaF, sodium fluoride; NaN3, Sodium azide; PBS, phosphate Buffered Saline; PMSF, Phenyl-Methyl-Sulfonyl Fluoride; PR, progesterone receptor; SDS, sodium dodecyl sulfate; TBS, Tris Buffer Saline; TBST, Tris Buffer Saline plus Tween-20; Tris-HCl, Tris-Hydrochloride.

Statement of Competing Interests

No competing interest is to be reported. This study was carried out in laboratory BMCN/UBBM, CNHU-HKZ and in the laboratory of Dr. Xiang-Xi Xu in the University of Miami Miller Medical School. No competing interest is to be reported by the authors. This research is collaboration between researchers in the team of Molecular Biomarker in Cancer and Nutrition of the Unit of Biochemistry and Molecular Biology (BMCN/UBBM) in the University Abomey Calavi, Medical doctors of the National University Hospital (CNHU-HKZ) in Cotonou, BENIN.

Ethical Statements

The Institute of Biomedical Sciences and Applications (CER-ISBA) approves this pilot study. Procedures followed in this study were in accordance of our institute ethical guidelines data obtained could not be traced to individuals. Written and signed Informed consent was obtained from the patient before sample collection and analyses.

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