Novel Anticancer Drug 5H-pyro[3,2-a] Phenoxazin-5-one (PPH) Regulates lncRNA HOTAIR and HOXC genes in Human MCF-7 Cells

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Breast cancer in women is the second most commonly cancer, after skin cancer. The percentage of mortality risk for breast cancer is 1 in 37 women (2.7%), which makes breast cancer represent the second cause of cancer death in women. Recently, new research based on previously published work in systemic chemotherapy and endocrine therapy field, have improved the incidence rates. The quinonic nucleus is common to many natural and synthetic products associated with anticancer and antibacterial activities, these compounds are typically DNA-intercalating agents. The Class I Homeobox genes (HOX in human and hox in mouse) control embryonic development and specific determination of positional identity anteroposterior axis of the human body. The HOX genes, are 39 transcription factors related to morphological, physiological disease. It has been demonstrated that any deregulation into the network is able to induce neoplastic transformation. Particularly, HOXC locus collaborating with lncRNA HOTAIR play a key role in breast cancer.

In this study, our group evaluated the chemical and metabolic stability of new anticancer molecule 5H-pyro[3,2-a] phenoxazin-5-one (PPH). In a recent paper, we have already demonstrated that a new and potent anticancer synthetic iminoquinone, the 5H-pyrido[3,2-a]phenoxazin-5-one (PPH), is able to inhibit a large number of lymphoblastoid and solid-tumor-derived cells at submicromolar concentrations.

Based on our previous research, we decided to analyze the cytotoxic effect and capability of PPH to control the lncRNA HOTAIR and HOXC locus gene expression in human breast cancer cells MCF-7, in order to demonstrate its role like potential new breast cancer antitumor drug.

Key words: HOX, HOTAIR, cancer, drug

Breast cancer represents the most commonly cancer in women, second only to skin cancer. The percentage of mortality risk for breast cancer is 1 in 37 women (2.7%), which makes breast cancer represent the second cause of cancer death in women[1]. Usually, the 61% of the pathological cases were diagnosed when the tumor was identified in the breast tissue, while a third of the cases were diagnosed when the disease was in advanced or metastases condition[1]. Recently, new researches based on previously published work in systemic chemotherapy and endocrine therapy field, have reduced the incidence rates. However, drug resistance, stem-like capability or stemness, still represent a reducing factor of the successful treatment. The patients with breast cancer recurrences, always show drug resistance. Therefore, new research will play a key role in order to better understand the molecular mechanisms that controls neoplastic transformation and resistance to the therapy[1]. The identification of new molecular targets and understanding their role in the progression and development of breast cancer metastases will open up new possibilities for the development of active molecules against breast cancer.

The quinonic nucleus is common to many natural and synthetic products associated with anticancer and antibacterial activities[2], these compounds are typically DNA-intercalating agents because of the ability of their large, planar polycycles to bind strongly between the base pairs through hydrogen bonds and π-stacking interactions[3,4]. They usually have side chains or sugar substitutes and basic nitrogens, which upon protonation further strengthen the DNA binding. Examples of quinone derivatives with antitumor activity include mitoxantrone, doxorubicin, mitomycin, streptonigrin and actinomycin D (AMD)[5-7]. All known quinonic DNA intercalators have the potential to disrupt the normal function of DNA, leading to cell death. This DNA damage can be caused either by the parent form or by its metabolic conversion to electrophilic or radical species[8]. In a recent paper, we described a potent anticancer synthetic iminoquinone, the 5H-pyrido[3,2-a]phenoxazin-5-one (PPH), able to inhibit a large number of lymphoblastoid and solid-tumor-derived cells at submicromolar concentrations. Interestingly, this compound also exhibits high inhibitory activity on the proliferation of wild-type and multidrug-resistant KB cells[9]. In fact, "multidrug resistance" (MDR), a phenomenon due to the prolonged use of chemotherapy agents, currently constitutes the major problem in cancer treatment. From these studies, we concluded that the excellent cytotoxic activity of PPH results from the intercalation at the middle 5'-GC-3' base pairs of the octamer [d(GAAGCTTC)]2[10] (Figure 1).

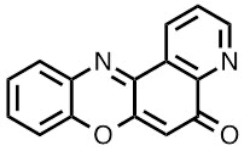

Figure 1: 5*H*-pirido[3,2-*a*] fenossazin-5-one (PPH)

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The Class I Homeobox genes (HOX) represent a highly conserved family of transcription factors characterized by the presence of the homeodomain. The HOX genes take part in the control of the cellular memory program through the close relationship with the Polycomb and Trithorax gene families and the control of the DNA-chromatin interaction. The collaboration between HOX genes and the Polycomb genes induces gene silencing, while the relationship between HOX genes and Trithorax genes, play a key role in the gene transcription[11]. A distinctive feature of HOX genes is that they are organized into specific and finely regulated groups, forming the most important identifiable network in the genome both functionally and physically[12] (Figure 2)

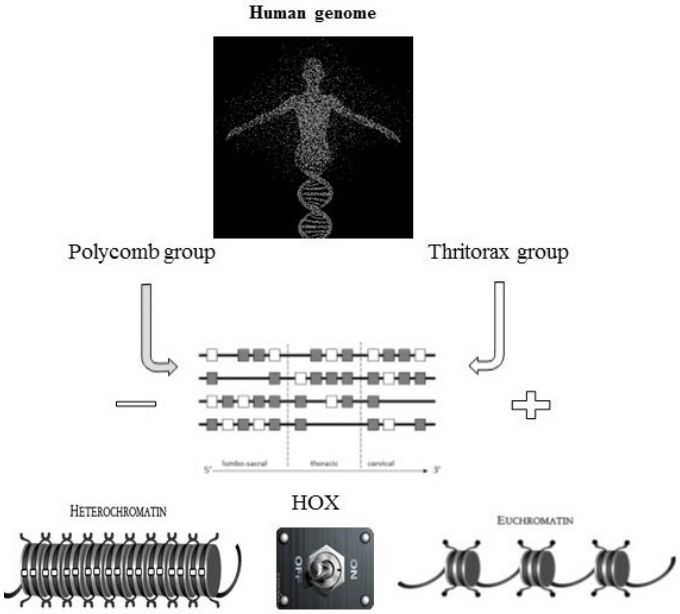

Figure 2: Gene memory program`

The HOX genes are activated during gastrulation, control embryonic development and specific determination of positional identity anteroposterior axis of the human body. The HOX genes, are 39 transcription factors organized in 4 clusters (HOXA, HOXB, HOXC and HOXD) located on 4 different chromosomes (HOXA chr-7, HOXB chr-17, HOXC chr-12, HOXD chr-2). The HOX proteins are characterized by the homeobox, a sequence of 183 nucleotides that encodes for a handyman of 61 amino acids, with an alpha-helix structure capable to bind a specific sequence of DNA activating or repressing genes target[13,14] (Figure 3). This transcription factor family, regulates different cellular processes such as cell division, adhesion, proliferation, differentiation and apoptosis. The analysis and comparison of HOX genes expression in human cells and tissues, has contributed to the development of specific patterns of expression; these studies have highlighted differences in the activity of the network HOX. Therefore, it has been possible to characterize healthy cells and tissues compared to pathological ones with particular reference to tumors structure[15-16].

It has been demonstrated that any deregulation into the network HOX, was related to morphological and physiological disease. Interestingly, change in Class I homeobox genes expression, can induce neoplastic transformation[17] confirming that HOX genes, play a crucial role during tumor progression, determines heterogeneity and metastases development[18,19]. Furthermore, the study of HOX network in several metabolic processes has contributed to better understanding specific

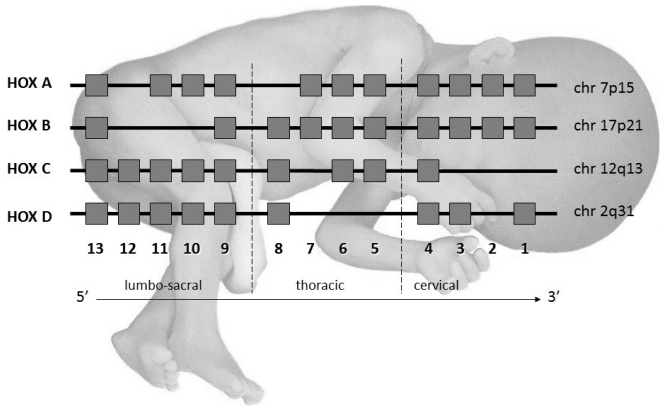

Figure 3. HOX gene network

aspects, typical of cancer cells metabolism and more generally to the biology of the cancer cell[20,21].

The role of HOX genes in breast cancer has already been shown, different HOX proteins were up or down regulated during neoplastic transformation. In details, 15 genes of the HOXA locus (A1, A2, A3, A4, A5, A6, A7, and A9) and HOXB (HOXB4, B5, B6, B7, B8, B9 and B13) have been identified and always overexpressed. Conversely, HOXC and HOXD loci showed different behavior: the HOXC5, HOXC6, HOSC8, HOXC11 and HOXC13 genes were constitutively expressed, while the HOXD locus genes showed a weak expression in cancer cells[22].

Recently, it has been demonstrated the involvement of long non-coding RNAs (lncRNA) in the determination of normal and pathological cellular processes and their ability to influence DNA and RNA expression and interaction[23]. HOTAIR (HOX intergenic antisense RNA) is one of the most important lncRNA associated with breast cancer and it is certainly the most representative; deregulation of HOTAIR, positively correlates with breast neoplastic transformation[16]. HOTAIR is lncRNA of 2.2 kb long and has been identified on the HOXC locus exactly between the HOX C11 and HOXC12 genes[24,25]. The HOTAIR is able to interact with the Polycomb 2 Repressive Complex, reprogramming the chromatin status and inducing metastasis[17]. In vivo experiments have shown that activating HOTAIR promotes the invasion of breast carcinoma cells[18].

In this study, our group evaluated the chemical stability of compound PPH. Moreover, compound PPH was also tested for vitro metabolic stability. Subsequently, we will analyze the cytotoxic effect and capability of Phenoxazinones to control the lncRNA HOTAIR and HOXC locus gene expression in human breast cancer cells MCF-7, in order to demonstrate its role like potential new breast cancer antitumor drug[26].

MATERIALS AND METHODS

Synthesis and characterization: 5H-pirido[3,2-a] fenossazin-5-one, (PPH) was prepared according to the reported literature, purified by HPLC and characterized by 1H NMR and 13C NMR spectroscopy co-confirmed data from literature[27].

Instrumentation and chromatographic conditions: The chemical and metabolic stability of PPH compound was monitored using a Nexera UHPLC system (Shimadzu, Kyoto, Japan) consisting of a CBM-20A controller, two LC-30AD pumps, a DGU-20 A5R degasser, an SPD-M20A photo diode array detector, a CTO-20AC column oven, a SIL-30AC autosampler.

The chromatographic separation was carried out on a KinetexTM C18 150 × 2.1 mm × 2.6 µm (100 Å) column (Phenomenex, Bologna, Italy). The optimal mobile phase consisted of 0.1% HCOOH/H2O v/v (A)

and 0.1% HCOOH/ACN v/v (B). Analysis was performed in gradient elution as follows: 0-3.00 min, 10-30% B; 10-11.00 min, 30-45% B; 11-12.00 min, isocratic to 95% B; 12.00-12.01 min, 95-5% B; then three minutes for column re-equilibration. Flow rate was 0.5 mL min-1. The column oven temperature was set to 40°C. Injection volume was 5 µL of PPH sample. The following PDA parameters were applied: sampling rate, 12.5 Hz; detector time constant, 0.160 s; cell temperature, 40 °C. Data acquisition was set in the range 190-800 nm and chromatograms were monitored at 240 nm. The calibration curve was obtained in a concentration range of 40-2.5 µM with five concentration levels and triplicate injection of each level were run. Peak areas were plotted against corresponding concentrations and the linear regression was used to generate a calibration curve (y = 0.00009x - 0.60210) with R2 values was ≥ 0.999.

Chemical and metabolic stability of PPH: The stability of the PPH compound under physiological condition was investigated. Briefly, PPH was solubilized in a phosphate-buffered saline (PBS, pH 7.4) and then its chromatographic profile was monitored at different time points (0, 24, 48, and 72 h) by RP-UHPLC-PDA experiments[28].

***In vitro* drug metabolism using liver microsomes of the PPH:** 25 µL of 5 mg/mL human (CD-1) microsomes (Thermo Fisher Scientific, Bremen, Germany) were pre-incubated with 0.625 µL of 100 µg/mL alamethicin, which forms pores in microsomal membranes, promoting access of substrate and cofactor to UGT enzymes. Then 2.5 µL of sample (2.5 mM) with 168 µL of 100 mM phosphate buffer (pH 7.4), 4 µL of 500 mM magnesium chloride were added, the mixture was incubated at 37°C for 5 min. The reaction has started by adding 50 µL of mix NADPH 10mM and UDP-GlcUA 20mM as cofactors (1:1 v/v). and carried out 37°C for 60 min in a Thermomixer comfort (Eppendorf, Hamburg, Germany). The reaction was stopped by the addition of 200 µL ice-cold methanol and then samples were centrifuged at 10,000 rpm at 25°C for 5 min (Eppendorf® microcentrifuge 5424, Hamburg, Germany). The supernatants were collected and injected in UHPLC. The control at 0 min was obtained by addition of the organic solvent immediately after incubation with microsomes[29]. As the positive control was used testosterone while the negative control was prepared by incubation up to 60 min without UDP-GlcUA/NADPH. The negative control is essential to detect problems such as non-specific protein binding or heat instability.

The extent of metabolism is expressed as a percentage of the parent compound turnover using the following equation:

$$\% \text{ Parent compound turnover} = 100 - \left[\frac{\text{concentration at 60 min}}{\text{concentration at 0 min}} \times 100\right]^{28}$$

AlamarBlue viability assay: MCF-7 cells (104 cells/well) were seeded in 96-multiwell plates. Cell proliferation of four replicate samples was assessed by alamarBlue assay (AB) at 7, 14 and 21 days. AlamarBlue assay uses a visible blue fluorogen probe resazurin, which is reduced to a red fluorescent compound (resorufin) by cellular redox enzymes of the mitochondrial respiratory chain. Viable cells continuously convert resazurin to resorufin, thereby generating a quantitative measure of viability and proliferation. The AB assay was performed according to the manufacturer's protocol (BioSource International, Camarillo, CA, USA)[30]. At predetermined culture intervals, 200 µL of Alamar Blue dye were added directly into culture media at a final concentration of 10 % and the plates were returned to the incubator for 4 h. As a negative control, AB was added to the medium without cells. The percentage AB reduction was calculated from the values of optical density at 540 and 590 nm using the manufacturer's formula[31].

RNA Extraction and Analysis: Total RNA was isolated from MCF-7 culture cells, using RNeasy Micro-Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. Samples were treated with RNase-free DNase (Qiagen GmbH) to prevent amplification of genomic DNA. One microgram RNA was subjected to cDNA synthesis for 1 h at 37°C using the Ready to go You-Primer First-Strand Beads kit (Amersham Biosciences cod. 27-9264-01) in a reaction mixture containing 0,5 mg random hexamers (GeneAmp RNA PCR Random Hexamers Set N808-0127 Applied Biosystems, Foster City, CA)[31].

RealTime-PCR (QRT-PCR): QRT-PCR will be performed using Taq-Man or technology (QRT-PCR StepOne). This assay uses a specific oligonucleotide probe, annealing between the two primer sites, which is labelled with a reporter fluorophore and a quencher. Cleavage of the probe by the exonuclease activity of Taq polymerase during strand elongation releases the reporter from the probe resulting in an increase in reporter emission intensity owing to its separation from the quencher. This increment in net fluorescence is monitored in real-time during each PCR amplification. The cDNA, will be used for real-time PCR performed in 48-well optical reaction plates with cDNA equivalent to 100ng RNA in a volume of 25 ml reaction containing Taqman Universal Master Mix (Applied Biosystem 4304437). Optimized concentrations of FAM-labelled probe and specific forward and reverse primers for HOXC9, HOXC10, HOXC11, HOXC12, HOXC13 and lncRNA HOTAIR (Applied Biosystem) from Assay on Demand. The results will be analyzed using a comparative method, and the values will be normalized to the GAPDH expression as endogenous controls[31].

RESULTS

Chemical and Metabolic Stability of PPH: High performance liquid chromatography (HPLC) analysis revealed that the PPH sample did not decompose after exposure to phosphate-buffered saline over a period of 72 h (Figure 4).

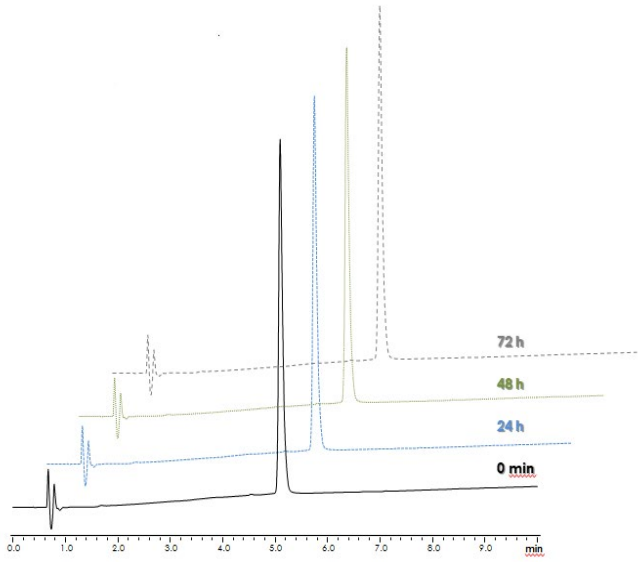

Figure 4: HPLC chromatograms obtained for PPH compound injected immediately after dissolution in PBS, after 24, 48 and 72 h. Spectra were recorded at 240 nm

Hepatic metabolism is the primary elimination mechanism for the majority of drugs, as well as for the other xenobiotics and endogenous compounds. As such, metabolism is a determinant of drug oral bioavailability, clearance, elimination half-life and therefore of dose and dosing frequency. Hepatic microsomes, derived largely from the smooth endoplasmatic reticulum, are commonly used as the enzyme source for the measurement of metabolic stability, as they contain the main drug-metabolizing enzymes cytochrome P450 (CYP) and UDP-

glucurosyltransferase (UGT)[32,33]. Metabolic biotransformation alters the rate of elimination of a compound from the body and can have a significant effect on the efficacy and safety of the agent[34] The UDP-glucuronosyltransferases (UGT) are a family of enymes that also reside in the endoplasmic reticulum, with UGT- catalyzed glucuronidation being responsible for the elimination of about 20% of marketed drugs[35]. PPH compound was tested for in vitro metabolic stability. The results showed that the molecule was generally stable in experimental conditions, showing a percentage of the parent compound turnover of 4.7 ± 1.1% in human (CD-1) microsomes[36] (Figure 5).

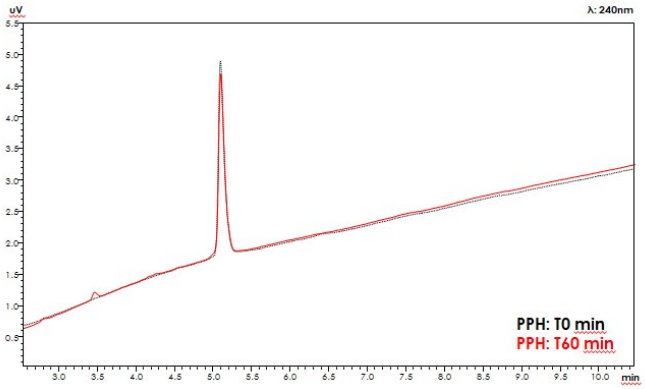

Figure 5: HPLC Chromatographic profiles of PPH sample before (black line) and after (red line) incubation with human microsomes

AlamarBlue Assay: The cell proliferation has been studied through AlamarBlue viability assay. The MCF-7 cells were treated with Phenoxazinones (PPH) and the proliferation was followed for 24h, 48h, 72h, 7, 14 and 21 days. The cells viability did not change during the period from 24h to 7 days of the experiments; while we observed some differences from 7 to 21 days, about the rate of viability of MCF-7 treated with PPH to different concentrations.

In details, the viability of MCF-7 was limited by PPH treatment at 0,04µ M compared to the control. Conversely, the proliferation of cancer cells didn't affect by PPH at 0,004µ M and 0,0004µ M compared to control (Figure 6, 7 and 8).

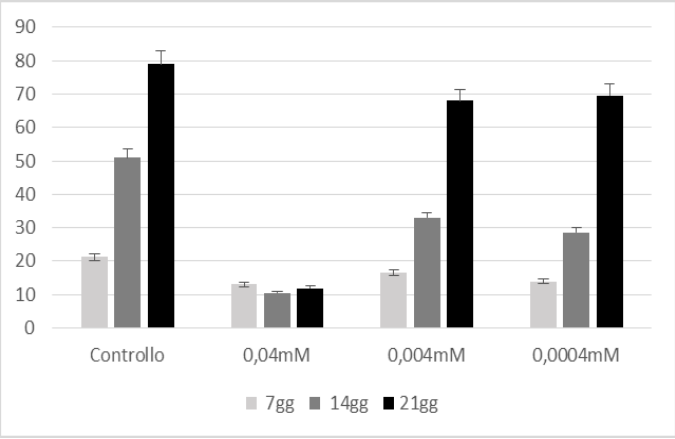

Figure 6: Alamar Assay in MCF-7 treated with PPH at different concentrations for 7-14-21days. The data are the average of five experiments (p <0.01)

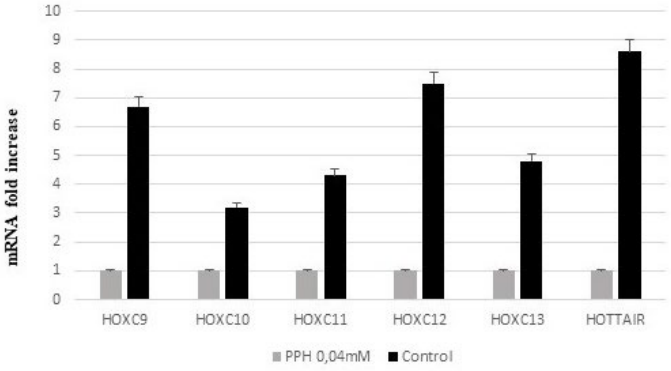

Figure 7: qRT-PCR in MCF-7 treated with PPH 0,04µM. The data are the average of five experiments (p <0.01)

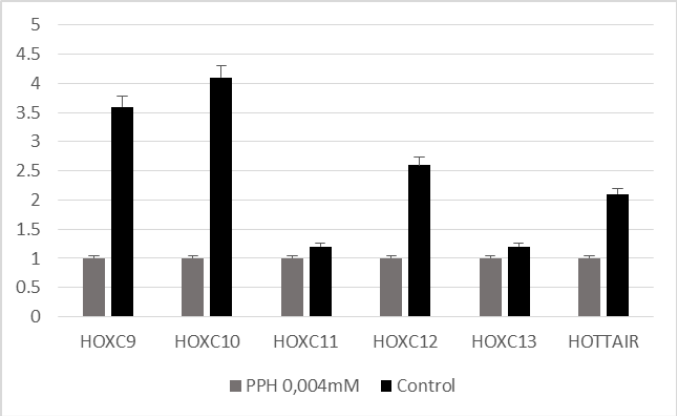

Figure 8: qRT-PCR in MCF-7 treated with PPH 0,004µM. The data are the average of five experiments (p <0.01)

qRT-PCR: The lncRNA HOTAIR, HOXC9, HOXC10, HOXC11, HOXC12 and HOXC13 genes were studied through quantitative gene expression analysis. qRT-PCR was set up in human cell carcinoma MCF-7 treated with PPH at the concentration: 0,04µ M, 0,004µ M, 0,0004µ M for 24h. The experimental conditions were performed following the material and methods described above. The Phenoxazinones was able to regulate in MCF-7, the expression of all genes object of the our study. Moreover, the rate of the gene expression was related to the different concentrations used in the experiments. In details, lncRNA HOTAIR was deregulated in MCF-7 exposed to PPH at 0,04µ M compared to control; while the differences in HOTAIR gene expression were decreased in the cells treated with PPH 0,004µM and 0,0004µM (Figure 9, 10 and 11). Considering the genes of the HOXC locus, our data demonstrated a significant difference in the rate of HOXC9, HOXC10, HOXC11, HOXC12 and HOXC13 gene expression in MCF-7 treated with PPH at 0,04µ M compared to MCF-7 treated with PPH 0,004µM and 0,0004µM.

In details, PPH was able to downregulate HOTAIR and HOXC genes and this capability was concentration dependent. Moreover, according with the literature, the difference in the HOTAIR gene expression is related to the regulation of HOXC locus exactly from HOXC9 to HOXC13

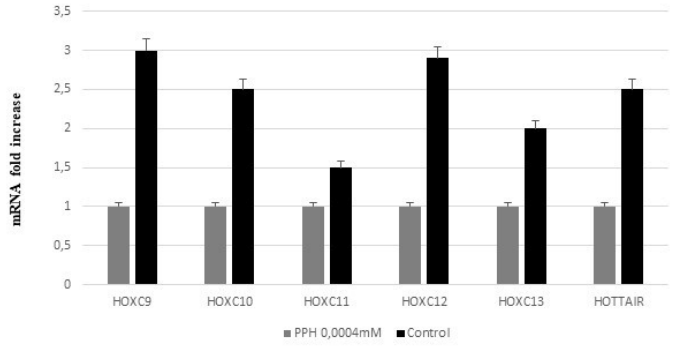

Figure 9: qRT-PCR in MCF-7 treated with PPH 0,0004µM. The data are the average of five experiments (p <0.01)

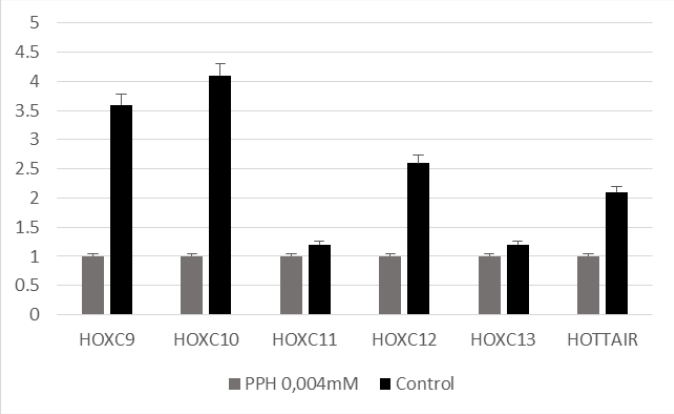

Figure 10: qRT-PCR in MCF-7 treated with PPH 0,004µM. The data are the average of five experiments (p <0.01)

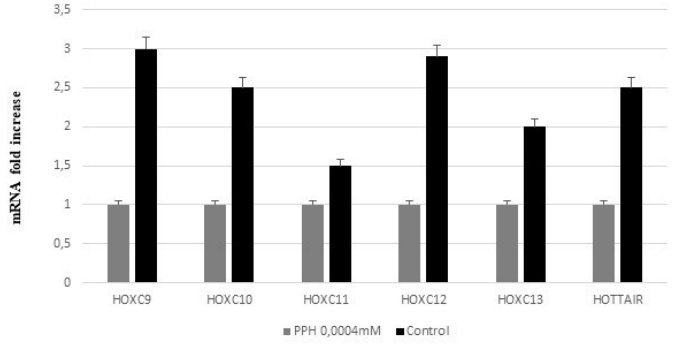

Fig. 11 qRT-PCR in MCF-7 treated with PPH 0,0004µM. The data are the average of five experiments (p <0.01)

DISCUSSION

Understanding the molecular mechanisms underlying the different malignant neoplasia, has been the basis for analyzing the new molecular targets to be used in cancer therapy, in order to improve healing rates. Breast cancer is the most important tumor among the cancer diseases affecting women.

In women with breast cancer with estrogen receptor (ER) and human epidermal growth factor receptor-2 (HER-2) positive, respectively, the use of drugs such as Tamoxifen and Herceptin (Trastuzumab) has induced an increase in cure rates[37]. Although the study of breast cancer has produced remarkable results, some aspects of this pathology are still poorly understood such as the identification of specific targets or molecular circuits that characterize specific tumor subtypes, or the reasons of drug resistance. The difficult to give an answer at these questions and understanding the mechanisms able to induce neoplastic transformation, tumor progression and development of metastasis, represents a challenge for researchers in order to achieve new molecular goals and identify new drug therapies in breast cancer treatment[38,39].

Recently it has been shown that non-coding RNAs play a key role in the development of the mammary gland[40] the expression of different lncRNAs is deregulated in breast cancer[41,42].

In details, lncRNA HOTAIR play a crucial role during Epithelial Mesenchyme Transition (EMT) process, is involved in pathogenesis and progression of several tumors. HOTAIR is 231 bases lncRNA able to interact with HOX genes; HOTAIR is transcribed from HOXC locus located at chromosome 12q13.13, in intergenic and anti-sense position to the flanking HOXC11 and HOXC12 genes[43-46]. HOTAIR is the first lncRNA that control the gene expression with trans mechanism. In details, it is able to block the transcriptional control of the HOXD locus on the chromosome-2, including the unique Polycomb Responsive Element (PRE) to date identified in the genome[47]. It has been shown that HOTAIR is one of the most characteristic factors of breast cancer[48-51]. Moreover, HOTAIR is an independent and specific molecular marker in case of poor diagnosis and metastasis risk[52]. Particularly, the expression of HOTAIR is upregulated in breast cancer, tumor progression and metastasis development compared to normal breast epithelium[53].

Recent study, suggests the involvement of specific molecular networks, regulated by HOTAIR, during the neoplastic transformation. In details, HOTAIR, as mentioned above, is a non-coding RNA located within the HOXC locus[54]. HOX genes represent a family of transcription factors that control embryonic development, the aberrant expression of one or more HOX genes is related to different morphological, metabolic and physiological pathologies, especially during neoplastic transformation[55].

Several HOX genes are deregulated in breast cancer, the HOXC cluster, from HOXC-9 and HOXC-13, it appears to be a very active area in breast cancer. In details, the overexpression of the HOXC-9 gene in MDA-MB-231 and MCF-7 tumor cell lines induce an increase of invasiveness but reduced proliferation by determining the transition from a proliferative to invasive cell phenotype[56]. The HOXC-10 is overexpressed in primary breast cancer and even more significantly in the metastatic processes that occur when chemotherapy treatment fails[57]. The HOXC11 has been shown to correlate with endocrine therapy resistance in breast cancer patients, primarily by interacting with Steroid Receptor Coactivator -1 (SRC-1) protein[58].

The HOXC13 gene is always hyper-expressed in breast cancer, particularly the co-expression of HOXC13 and HOTAIR has been demonstrated during the neoplastic transformation, in the metastatic process and this process was correlated with a high rate of poor prognosis[59].

The difficulty in determining new therapeutic strategies for this neoplasia, is related to the fact that breast cancer is basically a type of tumor with a high degree of genetic diversity and has many different molecular subtypes. Therefore, in order to understand the pathogenesis and tumor progression, it is necessary to identify common specific molecular target for classifying different tumor sub-types[60].

Previous studies about the analysis of HOX gene expression in breast cancer in tumor cell lines have found that these transcriptional factors play a key role in the pathogenesis of this neoplasia[61-63,64].

In this study, our group has synthesized a new molecule 5H-pyrido[3,2-a] phenoxazin-5-one (PPH) for using in breast cancer therapy. We decided to evaluate the ability of PPH, to regulate the activity of the HOXC locus genes (from HOXC-9 to HOXC13) and the lncRNA HOTAIR, in MCF-7 tumor cell lines.

In the first phase, we verified the metabolic stability of PPH the results showed (Table 1) that our molecule has a high degree of stability under the expected experimental conditions. Subsequently, we verified, through the AlamarBlue viability test, the cytotoxicity of the molecule at different concentrations. Our outcomes have demonstrated that MCF-7 cells, treated with PPH at different concentrations does not undergo viability change during 7 and 14 days; while the cells viability, was slow down after 21 days of treatment with PPH compared to control. Furthermore, the cell behavior showed drastic changes related to the PPH concentration. In details, the MCF-7 viability treated with PPH at 0.04mM stopped at 7 days around 10% compared to the cells exposed to PPH at 0,004µ M and 0,0004µ M (Figure 6).

***In Vitro* Drug Metabolism Using Liver Microsomes**			
Sample	**Regression curve**	**R^2**	**Metabolic stability using liver microsomes supplemented with NADPH AND UDP-GlcUA**
PPH	y = 0.00009x - 0,60210	0.99947	4.7± 1.1

Table 1. Metabolic Stability

The data relating to the analysis HOXC9, HOXC10, HOXC11, HOXC12, HOXC13 and HOTAIR gene expression were in agreement with scientific literature and in particular allow to highlight the ability of PPH to act as a positive regulator of these genes and its action has been always concentration dependent.

In details, the HOXC-9 gene expression was silenced in MCF-7 treated with PPH at 0,04µ M compared to MCF-7 control. The data of HOXC-10, HOXC-11, HOXC-12 gene expression, exhibited similar behavior to that described for HOXC-9; In particular, the HOXC gene expression, was always inhibited by the presence of PPH at the concentration of 0,04µ M, into the plate of MCF-7 cells (Figure 7).

Different mention deserves HOXC13 gene. It has been interesting to study the HOXC-13 behavior that was similar to previous genes, but we observed that, HOXC-13 gene expression, was strictly linked to the trend of HOTAIR gene expression. In fact, the HOTAIR and HOXC-13 behavior seems to be related and dependent to the PPH concentration. In fact, the silencing effect induced by PPH at the 0,04 µM was demonstrated by the regulation of the HOXC13 and HOTAIR gene expression in the MCF-7 un-treated (Figure 7). Conversely, we have observed that the HOXC9, HOXC10, HOXC11, HOXC12, HOXC13 and lncRNA HOTAIR gene expression was decreased in MCF-7 un-treated with PPH at 0,004 µ M and 0,004 µ M, lining up MCF-7 treated with MCF-7 control, confirming that PPH was able to inhibit the gene expression and this process is concentration dependent (Figure 8 and 9).

CONCLUSION

In conclusion, our data demonstrated that the new molecule synthesized in our laboratories, 5H-pyrido [3,2-a] phenoxazin-5-one, plays a key role in the regulation of genes strictly involved in the neoplastic transformation of breast cancer, such as lncRNA HOTAIR and a part of the HOXC locus. Particularly, our outcomes have shown that the expression of the HOXC genes object of our study was significantly reduced in MCF-7 cells treated with PPF, compared to the control.

Furthermore, we have shown that our molecule is extremely stable as demonstrated by the metabolic resistance test.

Considering this as a preliminary study, further research will be needed to make our molecule more efficient in counteracting the pathogenesis of breast cancer.

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