HOX transcript Antisense Intergenic RNA Role in Breast Cancer; Review Article

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Abstract

Genomic and transcriptomic analyses have revealed that as much as 85% of the human genome is transcribed. Non-coding regions of the genome were considered nothing more than transcriptional noise but now it is believed that they have a larger and more diverse role in biological processes than initially anticipated. Non-coding RNAs (ncRNAs) can be roughly classified into two groups based on their size. One group includes short RNAs less than 200 nucleotides (nt) in length, such as microRNAs (miRNAs) as well as, tRNA and piwi-interacting RNAs (piRNAs). The other group includes long ncNAs (lncRNAs) of around 200 nt or more. Cancer is a condition where gene expression is aberrant. The study of genetic background of cancer has revealed that the majority of the cancers are attributed to non-coding regions of the genome. Recent developments indicate that several cancer loci are transcribed into lncRNAs and that these transcripts play key roles in tumor genesis. LncRNAs contribute to cancer development through diverse mechanisms. For example, lncRNAs interact with nucleic material and protein molecules and/or their combinations and act as an essential regulator of chromatin organization, transcriptional and posttranscriptional regulation. Their misexpression confers the cancer cell potential to initiate tumor growth, and metastasis.

Keywords: Long non coding RNA

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Introduction

Genomic and transcriptomic analyses have revealed that as much as 85% of the human genome is transcribed . However, the majority of RNA transcripts are non-coding. Non-coding regions of

the genome were considered nothing more than transcriptional noise but now it is believed that they have a larger and more diverse role in biological processes than initially anticipated ***(1).***

Non-coding RNAs (ncRNAs) can be roughly classified into two groups based on their size. One group includes short RNAs less than 200 nucleotides (nt) in length, such as microRNAs (miRNAs) as well as, tRNA and piwi-interacting RNAs (piRNAs). The other group includes long ncNAs (lncRNAs) of around 200 nt or more ***(2).***

The term lncRNA itself is a very broad term conveniently ascribed to a transcript greater than 200 base pairs in length that does not appear to code for protein. In fact, several early lncRNAs have recently been shown to code for small proteins. Thus, one size fits all designation of lncRNA has been questioned and other designations have been suggested including the term transcripts of unknown functions (TUFs) or transcriptionally active regions (TARs). For now, lncRNA remains the accepted convention. The biological functions of lncRNAs are now starting to be understood, and critical roles for lncRNAs have been identified in nearly every biological system studied ***(3***).

lncRNA biogenesis and transcription

lncRNAs are similar to mRNAs in that many, but not all lncRNAs, are processed, 5′ capped, and polyadenylated ***(4).***lncRNAs are typically classifiedon the basis of their position relative to gene loci encoding protein-coding mRNA and enhancer regulatory elements of genes as either:

1) Overlapping when a protein-coding genes is encompassed by the intron of a lncRNA,
2) Bidirectional or divergent when the lncRNA and nearby protein coding gene are transcribed on opposite strands,
3) Intronic when the entire sequence of the lncRNA falls within the intron of a protein-coding gene,
4) Intergenic when a lncRNA sequence falls between two genes as a distinct unit, and
5) Sense or
6) Antisense if the lncRNA is mapped between one or more exons of another transcript on the same (sense) or opposite (antisense) strand ***(5).***

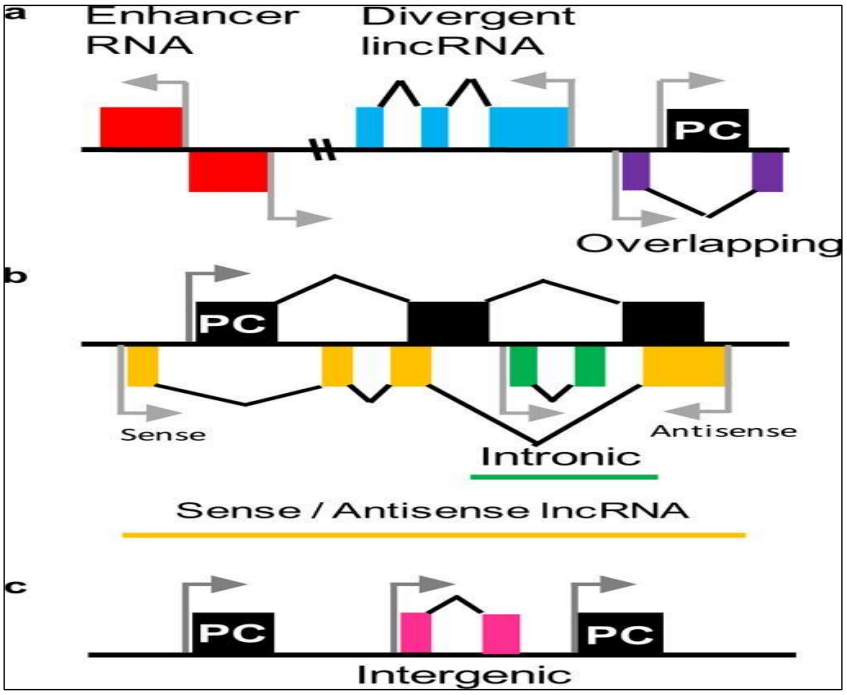


Figure (1): Long, non-coding RNA and enhancer RNA architecture.

(A) Enhancer RNAs (red exons) are transcribed in one direction (1d; unidirectional eRNA) or two directions (2d; bidirectional eRNA) in close genome proximity to known protein-coding genes. Divergent lncRNAs (blue exons) are transcribed in the opposite direction from known protein coding genes. Overlapping RNAs (purple exons) encompass the entirety of protein-coding genes in an intron region of the full-length lncRNA transcript. (B) Intronic lncRNAs (green exons) fall within an intron of a protein-coding gene. Sense and antisense lncRNAs (golden exons) are transcribed along the same (sense) or opposite (antisense) strand of the protein-coding gene. Exons from antisense lncRNAs are often partially shared. (C) Intergenic lincRNAs (pink exons) fall between known protein-coding genes and often regulate expression of these nearby mRNAs. (A–C) Gray arrows indicate direction of gene transcription. Square boxes represent gene exons that are spliced to form mature transcripts for the indicated RNA species. PC = protein-coding gene *(6)*.

lncRNAs have been associated with diverse functions. Their biological contributions have been seen in the form of:

1) Regulators of transcription in cis or Trans;

2) Modulators of mRNA processing, post-transcriptional control and protein activity; and

3) Organization of nuclear domains *(7)*.

Despite the elucidation of potential mechanistic roles, the biological relevance of the vast majority of lncRNA remains uncertain. In fact, their intricacy relies not only on their functional switch but also in their ability to be tissue/cell-specific.

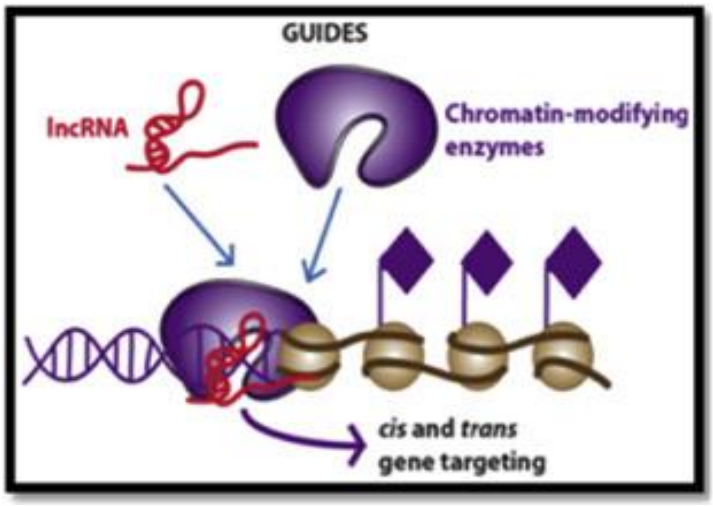
Furthermore, lncRNA that are detected overall with fewer than 1 copy per cell may appear abundant in certain types of cells or even in specific nuclear compartments, adding yet another layer of complexity ***(8)***.

Mechanism of action:

1. LncRNAs as chromatin regulators:

The number of lncRNAs with described functions is steadily increasing, and most of the reports revolve around their regulatory capacity. LncRNAs often function as important cis and trans-acting modulators for the expression of protein-coding genes ***(9)***.

LncRNAs can mediate epigenetic modification by recruiting chromatin-remodeling complex to a specific chromatin locus.



Figure(2): LncRNA-mediated transcriptional regulation. Interaction with and recruitment of chromatin-modifying enzymes (e.g., histonemethylases, acetylases, and deacetylases) to the target gene locus***(10)***.

LncRNAs can directly interact with chromatin-modifying enzymes and nucleosome-remodeling factors. The chromatin-modifying enzymes catalyze covalent changes of histones or DNA on the chromatin to affect the expression of genetic information. LncRNAs are known to associate with many histone- or DNA-modifying enzymes, including Polycomb Repressive Complex (PRC) MLL/TrxG complex, histone demethylase LSD1, DNA methyltransferase DNMT1, and DNA demethylation regulator GADD45a ***(11)***.

lncRNAs are more tissue-specific in contrast to the chromatin-regulating or epigenetic machinery that tends to operate widely in many tissues. Targeting lncRNAs may therefore result in better tissue specificity with lower general toxicity ***(12)***.

2. Transcriptional regulation:

LncRNAs act as co-factors to modify the activity of the transcriptional factor.Models of transcriptional regulation include the bridging scaffold model in which lncRNA may function by interacting with the same set of molecules, forming a scaffold for a protein complex that bridges the enhancer-like element and the promoter of a coding gene. Also, the tethered scaffold model may be involved, in which the lncRNA must be able to target this complex to a specific DNA sequence. Thus, the function of the lncRNA is to provide specificity to the chromatin modifying enzymes, acting as a tethered scaffold ***(13).***

3.Post-transcriptional regulation

The ability of ncRNAs to identify complementary sequences allows some specific interactions capable of regulating post-transcriptional processing of mRNAs like capping, splicing, editing, transport, translation, degradation, and stability at various control sites ***(10).***

LncRNAs in cancer

Cancer is a condition where gene expression is aberrant. The study of genetic background of cancer has revealed that the majority of the cancers are attributed to non-coding regions of the genome. Recent developments indicate that several cancer loci are transcribed into lncRNAs and that these transcripts play key roles in tumor genesis. LncRNAs contribute to cancer development through diverse mechanisms. For example, lncRNAs interact with nucleic material and protein molecules and/or their combinations and act as an essential regulator of chromatin organization, transcriptional and posttranscriptional regulation. Their misexpression confers the cancer cell potential to initiate tumor growth, and metastasis ***(14)***

Long ncRNAs play a pivotal role in various cancer types including breast cancer. Abnormal expression of lncRNAs contribute significantly to cancer initiation and progression in breast cancer ***(14).***

HOTAIR

HOX transcript Antisense Intergenic RNA (HOTAIR) is a spliced and poly- adenylated lncRNA with 2.2-kb. It is transcribed from the antisense strand of the Homeobox C (HOXC) gene cluster which is flanked by HOXC12 and HOXC11 on chromosome 12 . It was identified in 2007. HOTAIR is transcribed by RNA polymerase II, spliced, polyadenylated and 5'-capped like protein coding genes **(*15*).**

HOTAIR does not show any stem loops suggestive of being a pre-miRNA. HOTAIR is preferentially expressed in posterior and distal sites of the human body. In an experiment that was done by ***He et al., (16)*** on 10 mammalian and 3 non- mammalian vertebrates genome, looking for matches to the 6 exons of HOTAIR and its two conserved domains, they reported a poor sequence conservation. HOTAIR is not found in non-mammalian vertebrates. Human HOTAIR

is comprised of 6 exons (from 1 to 6) ***(16)***. Although HOTAIR RNA does not encode any proteins, it is important in gene regulation by modifying chromatin structure ***(17)***.

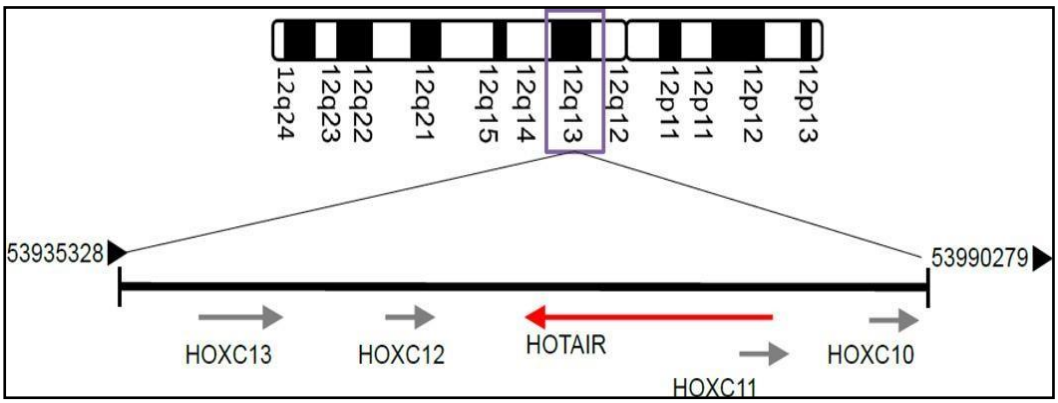


Figure (3): Genomic location of HOTAIR (*15*).

Mechanism of action of HOTAIR

Regulation of chromatin dynamics and inducing gene silencing via interaction of histone methylase (PRC2) and histone demethylase (LSD1).

Although there was no significant effect on HOXC cluster genes transcription; present in chromosome 12; where HOTAIR is actually coded, HOTAIR leads to transcriptional activation of HOXD locus genes present in chromosome 2, including HOXD8, HOXD9, HOXD10 and HOXD11. This indicates that HOTAIR regulates gene expression in trans fashion. It was the first lncRNA to be discovered to regulate gene expression in trans fashion way ***(15)***.

H3K27-methylation (H3K27-me3) is well recognized as the hallmark of gene silencing, introduced by histone methyl-transferase EZH2 (Enhancer of Zeste homolog 2), which is a member of PRC2 **(*15*)**. The core PRC2 complex comprises four components: EZH2, SUZ12 (suppressor of zeste 12 homolog), EED (embryonic ectoderm development) and RbAp46/48 (retinoblastoma associated protein 46/48) (also known as RBBP7/4, retinoblastoma binding protein 7/4). HOTAIR knockdown results in a significant loss of H3K27- trimethylation marks at the HOXD locus and loss of occupancy of SUZ12 in the HOXD loci. Occupancy levels of SUZ12 and H3K27-trimethyl marks were not affected at the silent HOXB locus, suggesting that HOTAIR is required to selectively target PRC2 complex to silence the transcription of HOXD locus ***(18)***

HOTAIR functions as a molecular scaffold and interacts not only with PRC2 but also with LSD1 (lysine specific demethylase 1A, also called KDM1) complex to regulate gene expression. LSD1 is a flavin-dependent monoamine oxidase, which demethylates lysines, specifically lysine 4 on histone 3 (H3K4) ***(18)***. So, HOTAIR facilitates recruitment of PRC2 and LSD1 multi-protein complexes at the target genes promoters, which induce H3K27-methylation and H3K4-demethylaytion

respectively and contribute to gene silencing. Through these functions,HOTAIR affects the expression of multiple genes involved in various cellular functions ***(17)***.

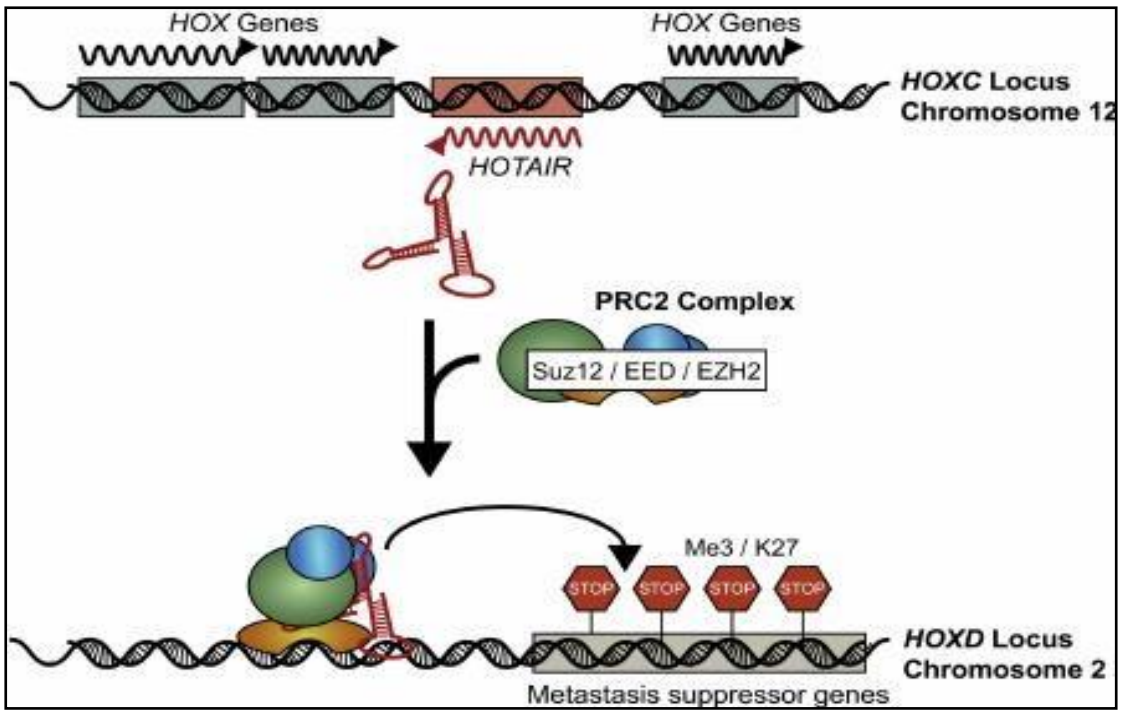


Figure (4): Mechanism of the gene-silencing action of HOTAIR ***(18)***

HOTAIR's Role in Breast Cancer

HOTAIR belongs to the first lncRNAs which have aberrant expressions that have been identified to associate with BC progression. It is able to interact with the main molecular pathways involved in BC carcinogenesis. Estradiol can regulate HOTAIR expression in ER+ BC cells for the presence of several EREs elements in its promoter ***(19)***.

Estradiol agonists, bisphenol-A and diethylstilbestrol are able to stimulate HOTAIR expression in in vitro and in vivo BC models ***(15)***. Moreover, both HOTAIR and breast cancer gene 1 (BRCA1) are able to bind the subunit of EZH2 (Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit) coordinating the PRC-dependent epigenetic regulation of the chromosome. Breast cancer gene 1 (BRCA1) is able to inhibit the binding of EZH2 to HOTAIR and its transfer on the promoter of PRC2 target gene HOXA9 in human BC cells and fibroblasts ***(20)***.

The promoter of the HOTAIR gene can also be bound by IRF1 (interferon

regulatory factor-1) able to induce its inhibition in BC cells. It is known that HOTAIR is also associated with an aberrant DNA methylation profile in cancer ***(21)***. In BC, the combination of HOTAIR overexpression and methylation status represents an important predictor of poor prognosis (***21***).

1)HOTAIR's Role in BC Metastatic Progression

Early studies highlighted the aberrant expression of HOTAIR in primary BC tumors with high metastatic potential and poor survival, suggesting HOTAIR as a powerful predictor of BC tumor progression ***(17)*.**

Some authors suggested that HOTAIR is an independent predictor of metastasis in ER+ patients. Other studies showed that the upregulation of HOTAIR can be considered a marker of metastatic progression only in ER- BC patients. These latest data have also been confirmed by in vitro investigations pointing out aberrant expression of HOTAIR, in particular in basal-like BC ***(22)***.

A recent study analyzed the in situ expression of HOTAIR in a large case series of TNBC patients and showed that high HOTAIR expression in tumor tissues is strongly correlated with lymph node metastasis, and it is directly associated with androgen receptor (AR) expression therefore potentially involved in the regulation of the AR pathway (***23***).

2) HOTAIR's Role in Epithelial-Mesenchymal Transition

Many studies have demonstrated that HOTAIR is also a critical modulator of EMT in BC .The treatment of BC cells with TGF-B1 leads HOTAIR upregulation and modulates the EMT process. This condition is reversed by induced downregulation of HOTAIR with a consequent reduction in the ability to form colonies. Recently, it was shown that Cancer-associated fibroblasts (CAFs) are able to promote BC metastasis via paracrine TGF-B1. The CAF-conditioned media of MCF7 and MDA-MB-231 BC cells strongly increases HOTAIR expression promoting EMT. Autophagy is also strongly involved in the modulation of EMT. HOX transcript antisense RNA (HOTAIR)-mediated autophagy could be a critical step in BC progression thanks to its ability to induce upregulation of metalloproteinases (MMPs) and B-catenin ***(19).***

The modulation of the EMT process as well as the consequent induction of metastatic processes is also strongly influenced by the activity of a series of microRNAs, especially in BC. The downregulation of miR-7 in BC patients is strongly associated with BC cancer stem cells and correlates with HOTAIR expression. The knockdown of HOTAIR leads to miR7 upregulation and reverts EMT and BC cancer stem cells proliferation (***24***).

A recent study has highlighted that HOTAIR is able to induce BC evolution by increasing the Bclw gene, belonging to the B-cell lymphoma 2 (bcl-2) family, via sequestering miR-206 at the post-transcriptional level **(*25*).**

Moreover, HOTAIR is able to physically interact with the miR34 promoter to silence miR34a in cancer stem cells (CSCs) from BC cells **(*25*).**

Recently, ***Han et al., (12)*** showed that delphinidin, an anthocyanidin, is able to suppress BC progression by upregulating the miR34a inhibition of HOTAIR and suppressing EMT through the downregulation of MMPs and the beta-catenin signaling pathway.

3) HOTAIR's Role as a Circulating Marker

The great diagnostic and prognostic potential of HOTAIR has also been supported by its detection in the blood of BC patients (***26)***.

The circulating level of HOTAIR from BC patients strongly correlates with the clinical stage, regardless of the molecular subtype (***24***).

Zhang et al., (***24***). showed that HOTAIR expression, analyzed in 148 plasma samples from BC patients, significantly correlates with ER and HER2 expression and with lymph node metastasis. In post-operative BC patients, a substantial reduction of its circulating level has been described (***24***). More recently, ***Tang et al., (27)*** showed that serum exosomal HOTAIR is a potent predictor of poor survival and drug response in BC patients, regardless of the molecular subtype **(*27*).**

4) HOTAIR in Breast Cancer Therapeutic Resistance

One of the main problems in breast cancer therapy is the establishment of an intrinsic or acquired resistance to treatment. Resistance to anti-tumor therapies can be linked to a variety of different factors such as genetic mutations, increased drug efflux, tumor heterogeneity, altered crosstalk between tumor cells and environmental factors or epigenetic changes related to the aberrant activity of many LncRNAs (***28***).

However, the knowledge of the mechanisms of resistance to the routine therapeutic agents in BC remains widely unknown. Long non coding RNA (LncRNAs) seem to be largely involved in drug responses for their ability to modulate the expression pattern of many oncogenes and oncosuppressor genes (***25)***.

(HOTAIR) aberrant expression has been widely described as a marker of drug resistance in different solid tumors. It can be involved in different resistance mechanisms related to the main routine treatments including radiotherapy, chemotherapy, and target therapies. (***25)***.

A. Radiotherapy Resistance

Radiotherapy is the leading therapeutic strategy for inoperable and locally advanced breast cancers.

Zhou et al., (29) investigated HOTAIR gene expression in five breast cancer tumor cell lines showing that the upregulation of HOTAIR in MDA-MB231 cells accelerates cell proliferation and enhances the resistance to radiotherapy. To investigate the mechanism controlling HOTAIR induced radio-resistance, the expression of HOXD10 (the translation of which is repressed by HOTAIR contributing to the acquisition of metastatic phenotypes) was analyzed. For the same purpose, the expressions of pBAD (Bcl2-associated agonist of cell death) involved in apoptotic pathway, and pAKT, involved in the cell proliferation pathway, were evaluated. The results showed that HOTAIR promotes the proliferation of BC cells during radiation therapy by targeting HOXD10 and the PI3K/AKT-BAD pathway **(*29*).**

Lately, it has been described that the expression of HOTAIR increases following ionizing radiation treatment. HOTAIR knockdown results in slower proliferation of BC cells, DNA damage accumulation, cell cycle arrest in the G2/M phase, and an increase in radiation-induced cell apoptosis. The radiosensitizing effects of HOTAIR silencing are related to the recruitment of miR-218, a ceRNA of HOTAIR, involved in repairing radiation-induced DNA damage and in apoptosis ***(30)***.

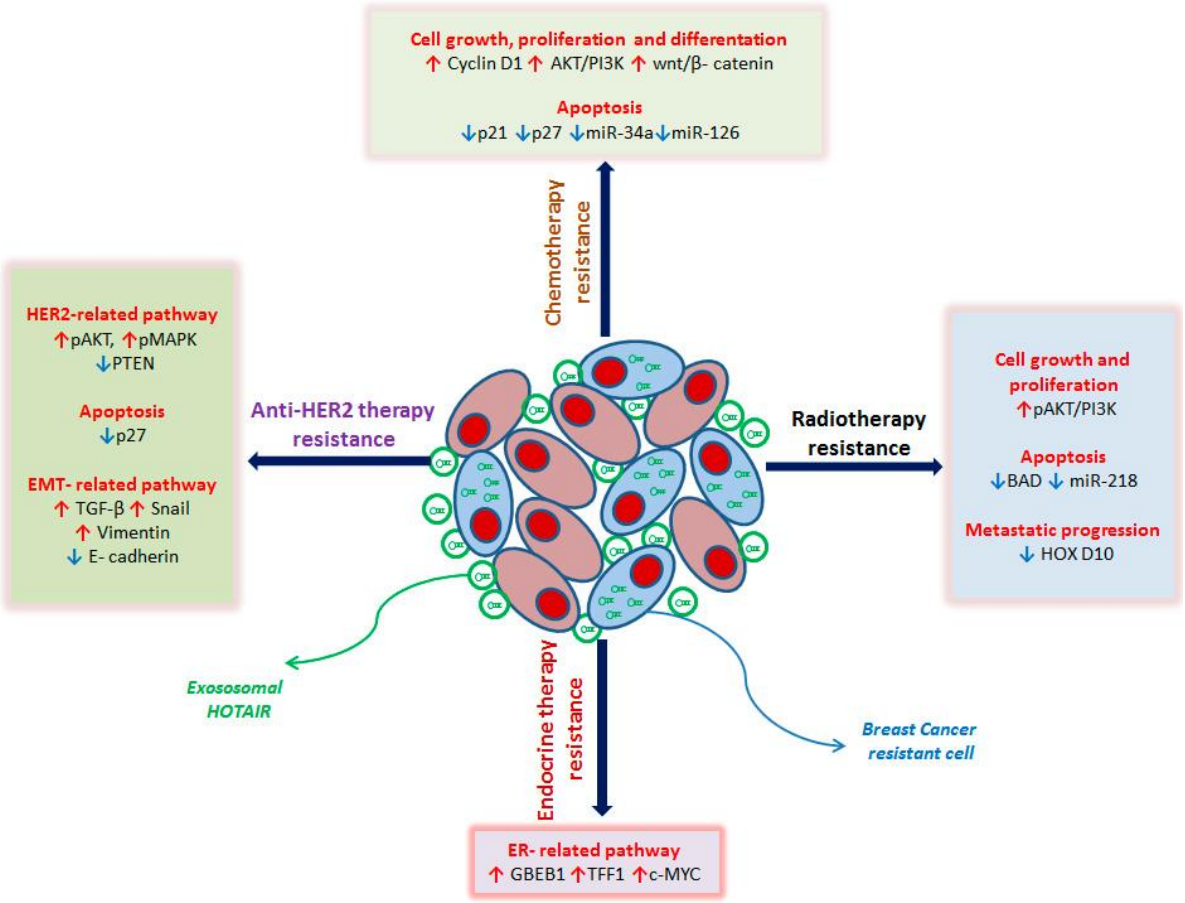


Figure5. Schematic representation of HOTAIR role in breast cancer (BC) drug resistance mechanisms with details of the main molecular pathways involved. In anti-HER2 treatment-resistant BC cells, the overexpression of HOTAIR leads to: (i) deregulation of HER2-related genes by upregulating the signal transduction pathway PI3K-Akt and downregulating the tumor suppressor gene PTEN with the consequent increase in proliferation, cell growth, and survival; (ii) inhibition of apoptosis by thedownregulation of cyclin-dependent kinase inhibitor p27; (iii) induction of EMT by TGF-_, Snail and Vimentin upregulation, and decrease in E-cadherin expression. In endocrine therapy resistant BC cells, the overexpression of HOTAIR leads to the repression of ER and the activation of ER-responsive genes, such as GREB1, TFF1, and c-MYC, promoting cell proliferation. In BC radio-resistant cells, the overexpression of HOTAIR leads to: (i) promotion of cell growth and proliferation by upregulation of the PI3K-Akt pathway; (ii) blockage of apoptosis by downregulating the pro-apoptosis gene BAD and miR-218, normally involved in the repair of radiation-induced DNA damage; (iii) induction of metastatic spread by

silencing of HOXD10, a metastasis suppressor gene. In chemo-resistant BC cells, the overexpression of HOTAIR leads to: (i) promotion of cell growth, di_erentiation, and proliferation by upregulating Cyclin D1, the PI3K-Akt pathway, and the wnt/_-catenin pathway; (ii) inhibition of apoptosis by downregulating cyclin-dependent kinase inhibitors p21 and p27, miR-34a and miR-216, both involved in promoting programmed cell death. The red arrows indicate the upregulated genes, the blue arrows the downregulated genes. HER2: human epidermal growth factor receptor 2, PI3K: Phosphoinositide 3-kinases, Akt: protein kinase B, PTEN: Phosphatase and tensin homolog, TGF-beta: Transforming growth factor beta 1, EMT: epithelial–mesenchymal transition, ER: Estrogen Receptor, GREB1: Growth Regulating Estrogen Receptor Binding 1, TFF1: Transcription Termination Factor 1, c-MYC: myelocytomatosis viral oncogene homolog, BAD: BCL2 antagonist of cell death, HOXD10 :Homeobox D10 **(31)**.

B)Endocrine Therapy Resistance

The antagonist of the estrogen receptor Tamoxifen is the most commonly used drug for ER+ BC patients, but the acquired resistance to the treatment represents the most important limitation for its use (***32***).

Xue et al. (33) showed that 37 lncRNA genes are repressed by estrogen and up regulated in tamoxifen-resistant MCF7 cells. HOTAIR is the main upregulated lncRNA in tamoxifen-resistant breast cancer and it is able to interact with ER, repressing it, but enhancing its transcriptional activity also in the absence of ligand. HOTAIR overexpression is able to induce ER-target gene expression such as GREB1, TFF1 and c-MYC in the absence of estrogen. Knockdown of HOTAIR strongly decreases tamoxifen-resistant MCF7 cell growth and inhibits the colony-formation abilities. These data suggest that HOTAIR is involved in tamoxifen-resistant cell growth and that this drug resistance may be reverted by targeting HOTAIR ***(33).***

Aromatase Inhibitors (AI) act blocking the enzyme aromatase, involved in the biosynthesis of estrogen reducing the growth of hormone-receptor-positive BC cells. AI are mainly used in postmenopausal women in whom it has better therapeutic effects than tamoxifen. Preliminary data performed on a large series of hormone receptor-positive early BC patients treated with AI, directly or after tamoxifen switch, showed that HOTAIR overexpression strongly correlates with clinic-pathological parameters, survival and AI resistance **(31)**.

C) Anti-HER2 Therapy Resistance

Trastuzumab is a humanized monoclonal antibody that binding HER2 receptor suppresses the formation of HER2 dimer interfering with downstream signaling pathways and promotes the inhibition of cell proliferation and apoptosis ***(34)***.

Resistance to trastuzumab is one of most clinic issues for HER2+ BC patients. A more recent study has showed that, in trastuzumab-resistant BC cell line, HOTAIR is overexpressed. In these cells, HOTAIR promotes the transition of tumor cells from G1 phase to S phase and inhibits the

apoptosis. To define the molecular mechanisms underlying the trastuzumab resistance mediated by HOTAIR, HER2 receptor signaling pathway related, PI3K/AKT/mTOR and MEK/ MAPK, have been analyzed in the sensitive and resistant BC cells. In resistant cells, HOTAIR overexpression is associated with the upregulation of p-AKT, p-MAPK and CyclinD1 and with the downregulation of tumor suppressor gene PTEN and cyclin-dependent kinase inhibitor P27, involved in the block of G1/S-phase transition. This leads to an increase in the cell growth, proliferation, survival and apoptosis. The knockdown of HOTAIR leads to the downregulation of p-AKT, p-MAPK and CyclinD1, and upregulation of PTEN and P27. This silencing is able to sensitize BC cells to trastuzumab blocking cell division at G0/G1 phases and promoting apoptosis. Moreover, in the resistant cells, the transcription and translation of TGF-B, Snail and Vimentin are up regulated while E-cadherin is down-regulated, promoting EMT. HOTAIR silencing reverts these results*(3)*.

D) Chemotherapy Resistance

Cytotoxic chemotherapy is largely used in routine therapeutic schemes both in advanced and early BC stages *(34)*. In particular, anthracyclines (mainly epirubicin and doxorubicin) are considered standard adjuvant therapy for patients with high-risk early BC. Along with anthracyclines, which are extremely cardiotoxic, taxanes (mainly docetaxel and paclitaxel) are the most active cytotoxic drugs in BC. Furthermore, fluorine derivatives (5-fluorouracil and capecitabine), methotrexate, vinorelbine, gemcitabine, and platinum derivatives (mainly cisplatin and carboplatin) represent the therapeutic alternatives for BC patients, alone or in combination with other drugs (*35*).

Tang et al., **(*27*)** analyzed circulating HOTAIR levels in the serum of 112 breast cancer patients before neoadjuvant chemotherapy (NAC) treated with different cytotoxic drugs to evaluate its predictive value. The study showed that high circulating HOTAIR levels strongly correlate with poor response to NAC.

Furthermore, a recent study showed that serum exosomal HOTAIR levels in BC patients 3 months after surgery are significantly reduced compared to levels before surgery, and a high level correlated with poor neoadjuvant chemotherapy **(*27*).**

Delphinidin is one of the main anthocyanidins and has strong anti-cancer properties, and it is able to suppress tumor transformation in breast cancer cells. Delphinidin is able to downregulate HOTAIR and simultaneously upregulate miR-34a, inducing apoptosis, in BC cells. Moreover, delphinidin treatment significantly decreases β -catenin, glycogen synthase kinase-3 β (Gsk3β), c-Myc, cyclin-D1, and matrix metalloproteinase-7 (MMP-7) expression. (HOTAIR) overexpression, in turn, can block the effect of delphinidin on the miR-34a and Wnt/ β -catenin signaling pathway in MDA-MB-231 cells, suggesting that delphinidin may potentially suppress breast carcinogenesis through the HOTAIR/miR-34a axis

(12)

5. HOTAIR as Therapeutic Targets in Breast Cancer

In recent years, having been strongly validated the clinic-diagnostic capabilities of lncRNAs, many therapeutic strategies have been suggested for targeting lncRNAs. Some lncRNAs have already been validated as potential therapeutic targets, with very encouraging results obtained on cell and animal models. Regarding HOTAIR silencing, the most used experimental approach is siRNA, able to deplete HOTAIR molecules both at the cytoplasmic and nuclear level **(*36*).**

***Gupta et al., (17*).** examined the effects of manipulating HOTAIR levels in several breast cancer cell lines. In particular, its silencing by siRNAs in MCF7, a cell line that expresses endogenous HOTAIR, decreases its capacity to invade Matrigel, a basement-membrane-like extracellular matrix ***(17*).** Knockout HOTAIR studies made it possible to validate the main role of HOTAIR in the modulation of cell proliferation, invasion, migration as well as in the apoptotic processes in BC models **(*19*).**

Bhan et al., (15) used a synthetic small interfering sense (siSENSE) oligonucleotide DNA complementary to HOTAIR transcript. The normal phosphodiester bonds of the HOTAIR-siSENSE DNA molecule were replaced with phosphorothioate linkage to minimize the nuclease digestion and enhance its in vivo stability. The HOTAIR siSENSE is able to silence specifically and effectively HOTAIR transcript levels in a dose-dependent manner.

(HOTAIR) silencing leads to apoptosis in MCF7 BC cells though upregulation of Bcl2 and BAD expression. Moreover, HOTAIR knockdown induces upregulation of its target genes HOXD10 and PCDHB5 **(*15*).** These data on MCF7 BC cells have been confirmed by the siRNA downregulation of HOTAIR or EZH2, a member of PRC2. This silencing is able to repress BC cells' proliferation, invasion, and migration and, at the same time, to promote apoptosis (***12***). (HOTAIR) siRNA in MCF7 BC cells is also able to increase mRNA levels of the luminal markers such as GATA3, KRT8, and E-cadherin and to reduce the basal marker as VCAN. (HOTAIR) expression in BC cells can be enhanced through prolonged and progressive exposure to TNF- β , a cytokine produced by the tumor microenvironment. The inhibition of p38 and SRC kinases, two mediators of the cell responses to TNF- β, can decrease HOTAIR expression and restore the expression of E-cadherin and KRT8 in MCF-7 cells (***22*).**

(HOTAIR) silencing in BC cells also shows a great impact on the modulation of EMT processes and in the self-renewal capacity of BC CSCs, being the majority of EMT/stemness genes regulated by HOTAIR. (HOTAIR) silencing leads to a downregulation of TGF- β, Snail, Vimentin, p-AKT, p-APK, and CyclinD1 and an upregulation of E-cadherin, PTEN, and P27, causing the inhibition of EMT in BC cells***(3)***.

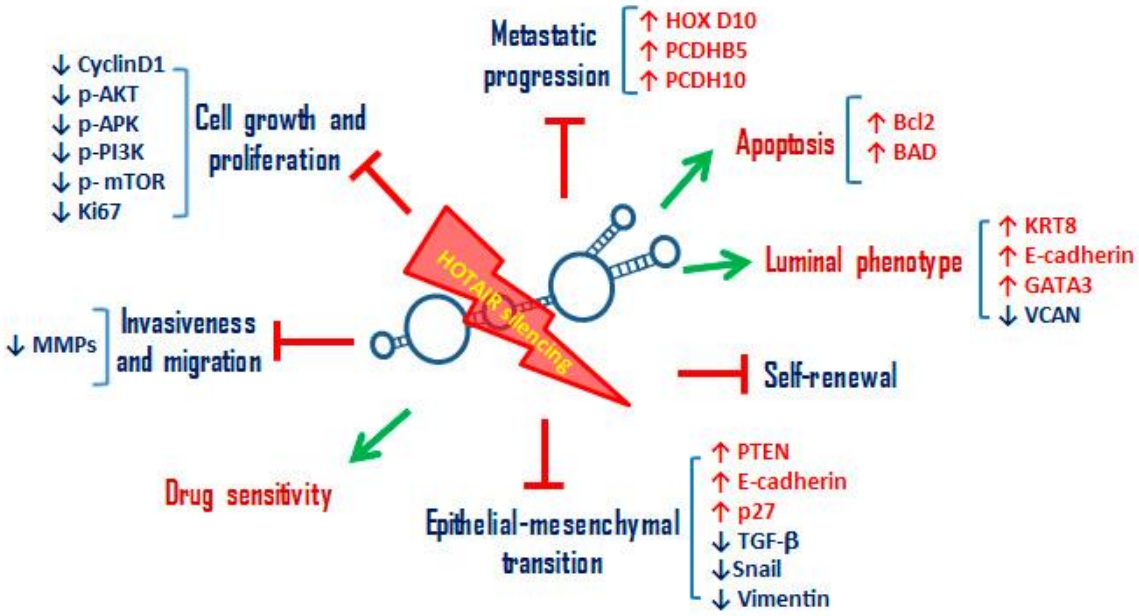


Figure 6. Schematic representation of the main cellular processes inhibited or activated by the silencing of HOTAIR in BC cells. HOTAIR knockdown in BC cells leads to the promotion of:

(i) sensitivity to radiotherapy, chemotherapy, hormonal therapies and anti-HER2 therapies; (ii) luminal phenotype acquisition with the upregulation of luminal cytokeratins (KRT8), of cell adhesion molecule E-cadherin, and of transcription factor GATA3, responsible of luminal epithelial differentiation in the adult mammary gland. Furthermore, HOTAIR silencing in BC cells leads to the inhibition of: (i) cell growth and proliferation by downregulating the main signaling pathways involved in these cellular processes, such as PI3K/AKT/mTOR and MAPK/ERK pathway, cyclin D1 and proliferation index Ki67; (ii) invasion and migration by downregulation of metalloproteinases; (iii) EMT by upregulating epithelial markers, such as E-cadherin, downregulating mesenchymal markers, such as Vimentin, and TGF-beta signaling; (iv) self-renewal, reducing colonosphere and mammosphere formation; (v) metastatic progression by upregulating metastasis suppressor genes, such as HOXD10, PCDHB5, and PCDH10. The green arrow indicates the activated processes, the symbol y the inhibited ones. KRT8: Keratin 8, GATA3: GATA Binding Protein 3, mTOR: mammalian target of rapamycin, MAPK: mitogen-activated protein kinase, ERK: Extracellular regulated kinases, PCDHB5: Protocadherin Beta 5, PCDH10: Protocadherin 10**(31)**.

Due to the fact of its important role in therapeutic resistance mechanisms, several studies have reported that HOTAIR downregulation is able to make BC cells sensitive to different therapeutic treatments.

Hu et al ., (30) showed that silencing of HOTAIR in MCF7 BC cells is able to reduce cell survival inducing apoptosis in response to ionizing radiation. Moreover, in HOTAIR knockdown cells ionizing radiation induces more DNA damage and cell cycle arrest than in control cells ***(30).***

In a doxorubicin-resistant BC cell line (DOXR-MCF-7), HOTAIR silencing decreases cell proliferation and induces apoptosis in BC cells reducing doxorubicin resistance and simultaneously determines a reduction of PI3K, AKT, and mTOR phosphorylation inhibiting the molecular pathway (*37*).

Similarly, in a trastuzumab-resistant breast cancer cell line SK-BR-3-TR, knockdown of HOTAIR sensitizes BC cells to trastuzumab ***(3)***.

No Conflict of interest.

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