Overview of long non-coding RNA and its role in breast cancer

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Abstract

The mammary glands are made of different cell populations that have great cellular plasticity, which is mainly regulated by epigenetic changes that allow the expression of specific transcripts needed depending on the stage of development (embryonic, puberty, pregnancy, lactation, involution, and postmenopause). Therefore, it is important to maintain strict regulation at each stage because aberrations in epigenetic regulation can lead to breast cancer. Among the epigenetic regulators are long non-coding RNAs (lncRNAs), which play important roles during the development of the breasts. Further, they can be used as biomarkers in breast cancer since their expression and emergence correlate with the patient's survival, as well as the aggressiveness of the tumor; the lncRNAs lead to epigenetic changes and the activation of signaling pathways, causing an accelerated rate of proliferation, metastasis, and evasion of the action of chemotherapeutic agents. Therefore, lncRNAs may be potential therapeutic targets. In this review, we show examples of lncRNAs that exert resistance on the different subtypes of breast cancer, through the activation of signaling pathways protein kinase b (AKT), the extracellular signal-regulated kinases (ERK), the mammalian target of rapamycin (mTOR), and (wingless/integrated [Wnt])/(beta-catenin). Some of these pathways are shared among the different subtypes, allowing cancer cells to activate the same pathways through different mechanisms, promoting tumor growth and resistance to various therapeutic agents. Furthermore, the mechanisms by which lncRNAs help acquire resistance and strategies to determine their function are presented in this review.

Key words: Long non-coding RNAs. Epigenetics. Genetic mutations. Resistance.

Introduction

Micro- and macro-anatomy of mammary glands suffer drastic morphological, anatomic, and epigenetic modifications during embryonic development, puberty, pregnancy, lactation, involution (ceasing of milk production), and postmenopause (Fig. 1). These modifications are caused by epigenetic indicators, which may be extreme conditions (environment and nutrition) or signals specific to development (differentiation)¹ which cause changes at a biochemical level through post-transcriptional modifications observed in chromatin's non-histone proteins²⁻⁴, long non-coding RNAs (lncRNAs)⁵, and histones (methylated – turning off or

acetylated – turning on target genes)^{2,6} (Fig. 1). These alterations lead to the remodeling of chromatin, allowing the activation or inhibition of transcription in certain genes, leading to morphological and functional changes. These modifications occur in the different cellular populations of the mammary glands.

Mammary glands are made up of adipocytes, epithelial, endothelial, and stromal cells, which converge and communicate simultaneously through hormones, growth factors, and cytokines, ensuring the correct organization of the tissue⁷ (Fig. 2).

The types of communication (activation of the different signaling cascades) which will be established

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Figure 1. Epigenetic changes caused by the stages of development in the breasts. To fulfill its function, the mammary glands present changes in their expression patterns, caused by biochemical modifications in their DNA, characteristic of each stage of development.

Figure 2. Communication between the different cellular populations of the breasts. The different populations of cells (adipocytes, endothelial, epithelial, and stromal) that make up the adult mammary glands constantly and simultaneously are in communication, through hormones, cytokines, and growth factors, to maintain the homeostasis of breast tissue.

amongst the heterogeneous cellular population of the breasts will depend on the development stage they are at (embryonic, puberty, pregnancy, lactation, involution and postmenopause). For example, during embryogenesis, morphogenesis depends on the signals originating from mesenchyme (supporting tissues which surround organs) and from the absence of hormones⁸. On the other hand, during the puberty-adulthood transition, the main signals of communication are those hormones which emanate from the pituitary gland and the ovaries. These stimulate the formation of the parenchyma (essential tissue of the organs) and allowing the ramification of the milk ducts, the development of the acinus, and the increase of the adipocytes (main component of non-lactating mammary glands). The latter, express aromatase enzymes which help in the conversion of androgens into estrogens, establish a paracrine communication with epithelial cells through the estrogen receptors alpha (ER). It is worth noting that epithelial cells release vascular endothelial growth factor, which allows the regulation of angiogenesis. Moreover, the proliferation, differentiation (during morphogenesis), and cellular death of epithelial cells are regulated by fibroblasts (another main component of mammary glands). These are in charge of maintaining, remodeling, and degrading the extracellular matrix (metalloproteinase)⁷. During pregnancy, progesterone and prolactin are secreted, promoting the formation of mammary alveolus, which will secrete milk during lactation⁸; while the involution of mammary glands leads to changes in the architecture of cellular populations, causing degradation and remodeling of the extracellular matrix. In addition, alveolar cells undergo an apoptosis. Even though the way this occurs is not fully elucidated, it is suggested to be a result of an inflammatory response which most likely is caused by the formation of α -lactalbumin (a component of milk)⁹. Finally, during the post-menopausal stage, the second mammary involution occurs, which is linked to a decrease in the functional activity of the ovaries, causing a remodeling of mammary glands including a decrease in mammary tissue and an increase in adipose tissue, which leads to a loss of function of this tissue⁹.

Mammary glands represent one of the more complex organs at a cellular level, due to the interactions (paracrine communication) between different cell populations. Moreover, each population brings its own cellular plasticity. Hence, the cells ought to maintain a fine epigenetic regulation, one which lets them activate the proper signaling pathways for each stage of development. Many of these processes are

Table 1. Similarities/differences between lncRNAs and transcripts that encode proteins

Similarities	Differences
Biogenesis (transcripts processed by DNA polymerase II)	They do not present an open reading frame
They present splice sites	They do not encode for proteins
Post-transcriptional modifications	They have a high rate of mutation in their genes
Their expressions are tissue specific and vary according to the stage of development	Low expression
They present hyperedition	

regulated by non-coding transcripts, among which are lncRNAs.

Characteristics of lncRNA

The number of genes which encode this kind of RNA remains uncertain, yet estimations suggest a range between 20,000 and 50,000 genes^{10,11}. These genes transcribe products >200 nucleotides of length. Those transcripts present characteristics very similar to messenger RNAs (mRNAs) regarding their biogenesis since the transcription process is carried out by the RNA polymerase II under a combination of different factors and transcription regulators, which join promoters and potentiators, present alternative splicing and post-transcriptional modifications (polyadenylation and hyperedition)¹²⁻¹⁴. In addition, its expression patterns just the genes which encode proteins and specific tissue and vary depending on the stage of development¹⁵. On the other hand, the differences between lncRNAs and mRNAs are the lack of an open reading framework, and its expression tends to be lower^{12,16}.

In addition, lncRNA genes have a high evolution rate concerning those genes which encode proteins since they evolve neutrally and the selection force exercised is low, indicating that many of these transcripts are not functional (Table 1). The amount of lncRNA sequences preserved by natural selection is lower than 5%¹⁷.

However, at the sequence level, lncRNAs do not present preserved regions. On the contrary, their secondary structure is well preserved, allowing them to maintain their function¹⁸. A clear example is provided by the lncRNA BORG (BMP/OP-Responsive Gene), which promotes the development of breast cancer in murine at an accelerated proliferation and metastasis rate, and when transfected in MCF-7 cells (breast cancer cellular line with a low BORG expression), changes

Figure 3. Genomic organization of long non-coding RNAs (lncRNAs). lncRNAs are classified according to their location in the genome; **A:** which can be present between two genes, which are called intergenic lncRNAs; **B:** or they may be located in introns (between two exons) of genes encoding proteins which are called intronic lncRNAs; **C:** They can also be located in the coding regions, that is, in the exons, and overlap in the same direction of the genes that encode proteins or in the opposite direction, these being called natural sense; **D:** or antisense; lncRNAs, respectively; **E:** On the other hand, bidirectional lncRNAs use the promoters of the genes that encode proteins but go in the opposite direction; **F:** and finally, the enhancing lncRNAs are located in enhancing elements (regulatory elements of the expression of genes that encode proteins).

in growth and inhibition of programmed death (apoptosis) can be seen¹⁹.

Based on their function, lncRNAs are divided into two kinds, constitutive or regulatory. lncRNAs with constitutive functions are constantly expressed and are involved in vital processes for the cell. lncRNAs with regulatory functions are expressed during development⁷. lncRNAs can be located in different parts of the genome, i.e. (a) intergenic lncRNAs exist between two genes which encode a protein; (b) intronic lncRNAs are produced from the intron of a gene; (c) natural sense lncRNAs are overlapping in the same direction with exons of genes that encode proteins; (d) antisense lncRNAs overlap in the opposite direction to the exons which encode proteins; (e) bidirectional lncRNAs share the same sequence promoters which encode proteins

but go in the opposite direction; and (f) enhancer lncRNAs come from enhancing regions located in genes which express proteins²⁰ (Fig. 3).

In the mammary glands, lncRNAs are in charge of transcriptional regulation, processing and maturing of mRNAs, and epigenetic regulation through the recruitment of chromatin remodelers (modifying methylation and acetylation patterns of histones). For example:

- Nuclear paraspeckle assembly transcript 1 (NEAT1) is an intergenic lncRNA, which is involved in the regulation and processing of transcripts, since it is an essential component for the formation of paraspeckles (compartments located in the nucleus), which are in charge of kidnapping proteins with transcriptional activity (transcription factors) and hyperediting mRNAs (change of an – A adenine for

- an – I inosine). This lncRNA is important for the morphogenesis and lactating process in mice²¹.
- ZNFX1 antisense RNA 1 is transcribed in the opposite direction to the gene, which encodes the ZNFX1 protein (zinc finger NFX1-type containing 1). Thus, it is an antisense lncRNA. This lncRNA functions as a tumor suppressor; it presents a high expression in mammary tissue and regulates alveolar development and differentiation of epithelial cells^{22,23}. Moreover, it modulates the assembly, production, and maturation of the ribosomes²⁴.
- Pregnancy-induced non-coding RNA is an intergenic lncRNA, which depends on the stimuli of the development for its expression (special and temporal) and interacts with the polycomb 2 repressor complex, inducing modifications in chromatin, mainly for the inhibition of transcript expressions which induce the differentiation of mammary alveolar cells and prevents milk production until delivery²⁵.

Therefore, alterations in the expression of lncRNAs, which maintain cellular homeostasis or that is in charge of the regulation of processes such as embryogenesis, puberty-adult transition, lactation, mammary involution, or postmenopause, lead to the initiation and progression of tumors which may result in breast cancer. These lncRNAs may be exclusive of one or be implicated in different subtypes of breast cancer²⁶.

Subtypes of breast cancer

Breast cancer is a very heterogeneous disease, which has allowed us to set a classification criteria based on transcriptional profiles (PAM 50) of genes that encode proteins, resulting in three subtypes of breast cancer; these three subtypes were found by Su et al. (2014). The same subtypes were found when making a classification based on lncRNAs. These findings can provide more personalized therapies.

Today, breast cancer is classified in the following main subtypes:

- Luminal (Luminal A and Luminal B)
Luminal A expresses estrogen and progesterone receptors and presents a low expression of Ki67. Luminal B expresses estrogen and progesterone receptors, negative or positive for human epidermal growth factor receptor 2 (HER2) and presents a high expression of Ki67.
This subtype responds well to hormone treatments, which may be aromatase inhibitors (enzymes in charge of the biosynthesis of estrogens), selective

modulators of ER (tamoxifen), and selective degraders of ER.

- HER2

HER2 presents an amplification and overexpression of the ERB oncogene (receptor) encoded by the HER2 gene. Following its activation, it promotes proliferation, differentiation, angiogenesis, invasion, and metastasis. Today, trastuzumab, which is a monoclonal antibody against ERBB2, is the approved therapy to treat this subtype²⁷.

- Triple negative or basal

Basal-type tumors, also called triple-negative breast cancer, do not present therapeutic targets, and only 20% of these tumors respond well to standard chemotherapy (therapies against RE)²⁸.

The role of lncRNAs in the different subtypes of breast cancer

It is of great importance to know the mechanisms and functions of breast cancer since they can be utilized as biomarkers to determine initiation, progression, and metastasis, or as potential therapeutic targets⁷ since they apply resistance toward chemotherapeutic agents, through the activation of the same signaling pathways in the different subtypes of breast cancer, using different targets. Next, there are some examples of lncRNAs (Table 2).

Urothelial cancer associated 1 (UCA1)

Its expression is regulated by the hypoxia-inducible factor 1-alpha (HIF-1A) transcription factor. This lncRNA presents a high expression in breast cancer, after its exposure to tamoxifen giving rise to the activation of the AKT/mTOR and Wnt/ β -catenin signaling pathways. Wnt/ β -catenin pathway activation prevents the degradation of β -catenin, translocating it to the nucleus, allowing the redistribution of the RE and the preservation of the characteristics of stem cells. Moreover, this lncRNA inhibits the expression of p27 (cell cycle regulator) and miR-18^a (microRNA 18s); the latter acts as a tumor suppressor when joined to the 3'-UTR (untranslated region) of HIF-1A (preventing it from joining its targets). Under normal conditions, miR-18a inhibits the Yes-associated protein 1 (YAP1), which positively regulates cell division cycle 42, which is a modulator of the cell cycle, and in breast cancer is involved in increasing proliferation, survival, and metastasis through the phosphatidylinositol 3-kinase (PI3K) pathway and mitogen-activated protein kinases

Table 2. Summary of the roles of lncRNAs involved in breast cancer

lncRNAs	Expression in breast cancer	Role in breast cancer	Pathways	Drug resistance
UCA1	Elevated	Maintenance of carcinogenic stem cells, increases proliferation, survival, and metastasis in cancer cells	AKT/mTOR Wnt/ β -catenin MAPK and HIF1A	Tamoxifen Trastuzumab
LINK-A	Elevated	Modifications in metabolism, greater energy disposition, and potency of tumorigenesis	Glycolysis and HIF1A	Endocrine drugs and trastuzumab
NEAT1	Elevated	Increases tumor size, metastasis, low survival in patients, EMT, avoids translation and hijacks transcripts that are responsible for regulating the cell cycle	HIF2A and WNT	Endocrine treatments and 5-fluorocyt
HOTAIR	Elevated	Low survival in patients; promotes migration angiogenesis, invasion, and metastasis in cells.	PI3K/AKT and HIF1A	Tamoxifen
BCAR4	Elevated	Helps cell proliferation and tumorigenesis	AKT/ERK	Tamoxifen
GAS5	Low	Formation of tumors and altering the cellular metabolism	AKT/PI3K	Tamoxifen
MA-Linc1	Elevated	Interrupts mitosis and destabilizes microtubules, promoting survival	Apoptosis and cell cycle	Taxol
MAPT-AS	Elevated	Stabilizes the transcript encoding TAU and prevents cell death	-----	Taxol

lncRNAs: long non-coding RNAs; UCA1: urothelial cancer-associated 1; NEAT1: nuclear paraspeckle assembly transcript 1; HOTAIR: hoX transcript antisense RNA; GAS5: growth arrest-specific 5; MAPT-AS: microtubule-associated protein tau antisense RNA; EMT: epithelial-mesenchymal transition.

pathways²⁹. Therefore, UCA1 plays an oncogenic role in breast cancer, allowing the creation of resistance against tamoxifen and trastuzumab (elevated expression of YAP1)^{30,31}.

Long intergenic non-protein coding RNA 1139 (LINK-A)

It is a cytoplasmic lncRNA with elevated expression in triple-negative breast cancer. It induces the HIF-1A signaling pathway under normoxic conditions, through the heterodimerization of epidermal-type growth factor bound to heparin dependent to the glycoprotein non-metastatic protein b (EGFR), giving rise to LINK-A recruitment, which interacts with the breast tumor kinase (protein tyrosine kinase) and leucine-rich repeat kinase, causing the phosphorylation of HIF-1A in its tyrosine 565 and serine 797, respectively. Phosphorylated serine 797 interacts with p300 (the regulator of the remodeling of chromatin), setting off the activation of the glycolysis pathway under normoxic conditions^{7,32} and enhancing tumorigenesis.

NEAT1

NEAT1 expression is regulated by the HIF-2A transcription factor, instead of HIF-1A (UCA1)³³; its high

expression is correlated with the size of the tumor and its metastatic potential, linked to a low survival rate. NEAT1 interacts with the forkhead box N3 repressor complexes and SIN3A (SIN3 transcriptional regulator family member A), which inhibit GATA3 (GATA binding protein 3 is a transcription factor which inhibits epithelial-mesenchymal transition [EMT]), allowing EMT⁷ in positive cells for the ER. Moreover, an elevated expression of NEAT1 increases the activity of the WNT signaling pathways, regulating ERs, and resistance to endocrine treatments such as the case of the UCA1 lncRNA. On the other hand, NEAT1 blocks the miR-211 function (tumor suppressor), allowing the expression of (high mobility group AT-hook [HMGA] which is non-histone proteins with transcriptional activity) and promoting EMT (miR-211 inhibits HMGA expression by binding itself in its region 3'), causing resistance to the 5-fluorouracil agent (analog of uracil, with an atom of fluorine in carbon 5)³⁴, which inhibits the normal functions of DNA and RNA³⁵. In addition, paraspeckles also play an important role in the proliferation and reduction of apoptosis in breast cancer, through kidnapping and editing (avoiding its translation) of (F11 receptor [F11R], which is an adhesion molecule) under conditions of hypoxia³³.

Hox transcript antisense RNA (HOTAIR)

It is a low survival biomarker and the regulation of its expression depends on the transcription factors HIF-1A, AP1 (jun proto-oncogenes), SP1 (Sp1 transcription factor), nuclear factor kappa B, and binding sites for estrogen response elements (EREs). The RE in the absence of estrogen interacts with ERE (ligand RE) and induces changes in chromatin through histone methyltransferase (MLL1 and MLL3), for the transcriptional activation of the HOTAIR gene, in cells positive to RE^{7,30}. Moreover, HOTAIR produces positive feedback when inducing the RE expression³⁶. Furthermore, in the presence of estrogen, HOTAIR expression decreases and growth regulating ER binding 1 expression increases. This is because the RE-estrogen complex induces a loop between the site of transcription start and enhancer of the HOTAIR gene, preventing its expression^{30,36}. HOTAIR alters the expression of its targets through its 5' and 3' regions, which join to PRC2 (transcriptional repression) and LSD1 (lysine demethylase 1A, which activates transcription), respectively³¹. Through its 5' and 3' regions, HOTAIR regulates the interaction of epithelial cells, increasing ATAXIN-1 degradation, which modulates E-cadherin transcription, promoting EMT, and also promoting metastasis and an increase of the HER 2 expression when bound to miR 331-3p (tumor suppressor)⁷. As for the induction of overexpression of HOTAIR by HIF1A in conditions of hypoxia, an elevated expression of AXL (tyrosine kinase receptor) is observed, promoting survival, migration, angiogenesis, invasion, and metastasis through signaling cascade of PI3K/AKT³⁷ in African women with breast cancer³⁸.

Growth arrest-specific 5

Reduces the efficacy of tamoxifen therapy through an increasing in phosphorylation of the ERBB2 and ERBB3 oncogenes, which are regulators of the AKT and ERK (1/2) signaling pathway³⁹. Furthermore, an amplification and overexpression of the HER2 receptor on the surface of the cells is exhibited, inducing the activation of the RE AIB-1 / SRC-3 coactivator and causing cell proliferation and tumorigenesis⁴⁰. In normal conditions (not cancer), GAS5 is a regulator of the metabolism, survival, and apoptotic stimuli; since it competes with the glucocorticoid receptor to bind to the glucocorticoid response elements⁴¹. Unlike other lncRNAs, this is subexpressed, correlating with a bad prognosis and creating resistance against trastuzumab.

Its low expression causes its target, miR-21, presents a high expression, inducing the formation of tumors through an accelerated proliferation and cellular invasion. Furthermore, this micro-RNA inhibits the expression of phosphatase and tensin homolog, which negatively regulates the AKT/PI3K pathway⁴².

Mitosis-associated long intergenic non-coding RNA 1 (MA-linc1) and microtubule-associated protein tau (MAPT) antisense RNA 1 (MAPT-AS1)

These lncRNAs evade the response to taxol, which is a stabilizing agent of the microtubules, preventing entry into mitosis and causing cellular death⁴³. MA-linc1 regulates the cell cycle, leading to the finalization of mitosis, through the destabilization of microtubules³¹. On the other hand, MAPT-AS1 is an antisense transcript of the MAPT gene, which encodes for TAU protein, which, in turn, competes with taxol to bind to microtubules. MAPT-AS1 binds to the MAPT transcript and stabilizes it to produce high levels of TAU⁴⁴.

Resistance acquisition mechanisms

Patients with cancer initially respond quite well to prescribe chemotherapeutic agents, but once they create resistance, survival prognosis lowers⁴⁵⁻⁴⁷. A key factor for resistance is the large intratumor heterogeneity since it is a combination of stem cells, progenitor cells, and its progeny, providing the tumor with very variable cell phenotypes, and can even be present in different subtypes of breast cancer. This idea is supported by the hypothesis that luminal subtypes and HER2 originate from progenitor cells committed to the luminal lineage, while the basal subtype comes from less differentiated stem cells, and in some cases, progenitor cells committed to luminal lineage can be precursors for the development of the basal subtype, through epigenetic and genetic changes^{28,48-50} (Fig. 4).

Other causes that add intratumor heterogeneity can be epigenetic changes and mutations in the genome, through parallel evolution, which allows the cells to present very diverse expression patterns that through positive selective pressure are selected and present in coding or non-coding regions (regulatory elements)⁵¹. These changes are transmitted to the progeny during cellular splitting⁵² (Fig. 5) promoting resistance toward chemotherapeutic agents.

Mutations of the genome lead to permanent changes of the DNA, while epigenetic alterations are transitory

Figure 4. Hypothesis of the development of the different subtypes of breast cancer. The cancer stem cells are capable of giving rise to slightly more differentiated stem cells such as progenitor cells committed to the luminal lineage and basal, allowing the development of the luminal-human epidermal growth factor receptor 2 and basal subtypes, respectively. However, sometimes, the progenitor cells committed to the luminal lineage are able to give rise to the basal subtype through epigenetic and genetic changes.

Figure 5. Mechanisms of resistance. Stem and daughter cells can use different mechanisms to develop resistance to various chemotherapeutic agents, among them **A**: parallel evolution, in which the stem cell passes on its mutations and epigenetic changes during cellular division to its daughter cells, whether stem cells or differentiated cells, which are capable of acquiring new mutations and epigenetic changes and transmitting them to their offspring. **B**: Another mechanism is the exosomes, which are compartments that carry LncRNAs and proteins in other molecules, and is able to be transported to other cells, allowing the development of resistance. **C**: Finally, there is hyperedition, of which we do not know much yet, but it is proposed that it can confer transcripts with certain characteristics that allow it to evade the action of chemotherapeutic drugs.

changes; the latter allows the cell to have a greater cellular plasticity without compromising the genotype. Studies have proven that resistance mechanisms can have a base in genetic or epigenetic changes.

Xue et al.⁵³ demonstrated genetic-based changes. Through a model of mice with xenografts derived from patients who were carriers of the B-Raf mutation (BRAFV600E allows the expression of the ERK pathway without external stimuli)⁵⁴. The model was administered different inhibitors of the ERK signaling pathway, and the emergence of extra chromosome material was observed through sequencing of a single cell and fluorescent hybridization *in situ*, which is believed to have arisen through parallel evolution shortly after treatment; the selective pressure of cells continuously treated with a single medication allowed the tumor cells to adapt while maintaining their intratumor heterogeneity⁵³. Regarding lncRNAs, HOTAIR is a clear example which is amplified in the genome, promoting resistance to tamoxifen⁵⁵.

Sharma et al.⁵⁶ proved a resistance with a base in epigenetic changes and also that this can be reverted. To do so, PC9 cells were incubated (cells derived from lung cancer, sensitive to inhibitors of EGFR-receptor epidermal growth factor) with inhibitors of the EGFR, inducing death in most of the cellular population, with only a small fraction surviving, which presented resistance toward the inhibitors. To determine whether these changes were genetic or epigenetic, the surviving cells were incubated with deacetylase histone inhibitors, causing cellular death after losing their resistance to the inhibitors of EGFR. On the other hand, Vaz et al.⁵⁷ proved that the environment induces epigenetic changes through exposure to bronchial epithelial cells (non-cancerous) to condensed cigarette smoke, causing epigenetic changes and inducing the transformation of wild-mutated KRAS (KRAS proto-oncogene, GTPase), revealing that progressive exposure to condensed cigarette smoke induces changes in the methylation of the DNA, and promoting pro-oncogenic changes. Concerning the lncRNAs, HOTAIR is a clear example of epigenetic changes caused by external stimuli or chemical agents like estradiol. The expression of HOTAIR is found to be elevated by its interaction with estradiol through its ERE-binding sites (in the promoter), modifying the structure of chromatin, and activating genes related to cellular proliferation, inhibition of apoptosis and EMT⁵⁸.

Another resistance mechanism is exosomes, which are vesicles that carry diverse RNAs and proteins. Xu et al.⁵⁹ proved that the cellular line MCF-7 (sensitive to tamoxifen), showed resistance to tamoxifen when treated with exosomes originated from the cellular line LCC2

(resistant to tamoxifen). Said exosomes presented a high expression of UCA1, causing proliferation, survival, metastasis, and resistance to tamoxifen and trastuzumab (Fig. 5)^{30,31,59}.

Moreover, lncRNAs can be hyperedited, which is suggested is also a key component of the transcriptional diversity in tumors⁶⁰. Hyperedition can lead to the loss of functions in lncRNAs⁶¹, mainly in those who have the function of tumor suppressors (F11R)³³. However, there is still a need for studies to determine if it plays an important role in resistance toward chemotherapeutic medications (Fig. 5).

Therefore, genetic changes like amplifications in the genome, together with the epigenetic alterations (which occur in genes which transcribe lncRNAs or in genes which are regulated by them), exosomes, and hyperedition give breast cancer cells distinctive characteristics (hallmarks). These provide them with advantages compared to normal tissue, such as the activation of signaling pathways related to cellular proliferation; evasion of growth suppressors, resistance to cellular death, cell replication capacity, induction to angiogenesis, activation of invasion (cellular metastasis), cellular metabolism reprogramming, and evasion to immune response⁶². Thus, cancerous cells present the same phenotype since the same signaling pathways are activated through synonym inductors, causing resistance to different therapeutic agents.

Strategies used to determine lncRNA functions

Studying the function of the lncRNAs implicated in breast cancer is critical, yet it remains a challenge because the bioinformatic tools do not allow us to determine the secondary structure and post-transcriptional modifications which it may suffer (hyperedition), altering the binding to its targets. Hence, the most viable methods nowadays are studies of loss/gain of function in *in vivo* or *in vitro* models.

Loss of function

RNA interference is utilized in this type of analysis to block the expression, but the inhibition is temporal. Therefore, the loss of function is transitory. Clustered regularly interspaced short palindromic repeats, associated protein 9 (CRISPR-Cas9), is a promising tool that allows the explanation of lncRNA functions, performing editions in the genome, either generating a partial or total deletion of the lncRNAs, or blocking the

expression from the alteration of the sequencings located between the promoter and the sequence of interest, through the insertion of polyadenylation signals⁶³.

Gain-of-function studies

The use of CRISPR-Cas9 also allows the overexpression of our sequence of interest due to the insertion of a strong promoter upstream the gene⁶⁴. However, the most used techniques for these studies are the use of plasmids or adenovirus carriers of the sequence of interest, being the overexpression transitory^{65,66}.

In vivo and in vitro models

Concerning the *in vivo* models, it is still difficult to establish an animal model, due to the high evolution rate of the primary sequence, among the species (activating targets and different pathways between species). Moreover, certain lncRNAs present in humans do not exist in other mammals. On the other hand, the use of cellular lines as an *in vitro* model does not provide us with accurate information, which can extrapolate patients since cellular lines present a high instability in its genomic and epigenetic architecture, which differs in large proportion to the tumor from which it derived. In addition, 3D cultures are needed and the use of diverse cellular populations that interact in normal conditions. Furthermore, the technologies used (expression microarrangements and sequencing) to analyze the function or targets of lncRNAs are very varied, causing inconsistencies among studies when analyzing the data⁷. Therefore, bioinformatics programs are needed, which will allow us to predict the function and possible modifications that lncRNAs may suffer.

Conclusion

lncRNAs can be used as therapeutic targets or biomarkers since they play an important role in the proliferation, metastasis, migration, and evasion of the actions of drugs in cancerous cells, promoting the aggressiveness of the tumor and causing a low survival rate. Moreover, lncRNAs intervene in the development of resistance mechanisms through the activation of the same signaling pathways in the different subtypes of breast cancer, either participating as an intermediary or stimulating the activation of transcription factors essential for the expression of the genes necessary for the activation of signaling pathways. Further studies of lncRNAs are needed, regarding the modifications performed by the hypermutation and determining whether

this process is linked to the development of resistance toward different chemotherapeutic agents and the presence of more aggressive breast tumors.

Conflicts of interest

The authors do not have conflicts of interest to declare.

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Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

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