Role of Long non-coding RNA Gene Expression in Breast Cancer

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Abstract

One of the most prevalent illnesses in women, breast cancer is thought to be a complex and diverse condition that continues to be a significant public health issue. Long non-coding RNAs have drawn a lot of interest lately because of the advancement of next-generation sequencing methods. Long non-coding RNAs have been shown in several studies to play significant roles in the formation of tumors. Recent studies have shown that a variety of long non-coding RNAs express aberrantly in malignancies, including breast cancer, despite the fact that the biological role and molecular processes of these RNAs are still unknown. With an emphasis on the many molecular functions of regulatory long non-coding RNAs that control cell proliferation, invasion, metastasis, and apoptosis, we have included the most recent research on long non-coding RNAs in breast cancer here.

Key words: Breast Cancer, public health, Long non-coding RNAs.

Breast Cancer Epidemiology:

Breast cancer (BC) ranks second in terms of global cancer incidence, accounting for an estimated 2.3 million new cases, or 11.6% of all cancer cases, and fourth in terms of cancer mortality globally, accounting for 6.9% of all cancer deaths, according to GLOBOCAN 2022 data. For women, breast cancer is the most common cancer diagnosed (24% of cases) and the leading cause of cancer-related deaths (15.5% of deaths) (1).

With an expected 22,700 occurrences in 2020 and an estimated 46,000 cases in 2050, breast cancer is the most prevalent cancer diagnosed in Egyptian women, accounting for 38.8% of all malignancies. An estimated 11% of people die from breast cancer, making it the second leading cause of cancer-related death after hepatocellular carcinoma (2). With a mean age of 50.4 years at diagnosis and 57% being premenopausal or perimenopausal, the Egyptian BC population was noticeably younger than their Western counterparts. Advanced stages were more common in Egyptian patients (positive lymph nodes: 70%, stage III disease: 45%, and stages T2 and T3: 55% and 21%, respectively). It's interesting to note that adolescent girls with BC had even more advanced stages when they first appeared; 48% of them were in stage III, compared to 49% and 27% in stages T2 and T3, respectively (2).

Breast cancer's intrinsic molecular classification:

In order to better comprehend the intratumoral heterogeneity in breast cancer, a number of molecular classifications have been established during the last 20 years using transcriptome and genomic

clustering. Nevertheless, in clinical practice, these intrinsic subtypes have not yet replaced the surrogate molecular subtype identified by immunohistochemistry(3).

Pioneer molecular classification at the start of this century. They analyzed 65 surgical slices of breast cancers from 42 patients and found four intrinsic molecular subtypes that were differentiated by gene expression profiling (GEP): basal-like, luminal A, luminal B, and HER 2 (Human Epidermal Growth factor receptor) gene overexpression (4).

Breast cancer that is Luminal (also known as Luminal A or B)

About 60% of breast cancers are of the luminal type, which is identified by the expression of ER. Type A of breast cancer is more uniform than type B, where: Luminal A has a low Ki67 proliferative index (< 20%), is ER positive, PR positive, and HER2 negative. Either Luminal B is (ER positive, PR negative / low, HER2 negative, Ki67 proliferative index high ;> 20%) Luminal B1, HER2 negative, Luminal B2, Ki67 proliferative index fluctuates, and HER2 positive (ER positive, PR positive or negative, HER2 positive). They typically appear in the early stages of cancer. Several assays, such as MammaPrint, endopredict & prediction analysis of microarray, PAM 50, and 21-gene recurrence score RS, have been created especially for this subgroup to offer recommendations for the best clinical care of individual patients. Molecular techniques have been most effective in predicting tumor biologic characteristics and treatment responses for this particular set of cancers so far (5).

In terms of the molecular alterations, this subtype of tumor is the purest. It is identified by fluorescence in situ hybridization (FISH), which shows HER2 gene amplification, or immunohistochemistry (IHC), which shows HER2 overexpression. ER negative, PR negative, HER2 amplified or overexpressed are all signs of HER2 enrichment. Luminal B2 has a variable Ki67 proliferative index and is HER2 positive (ER positive, PR positive or negative, and HER2 positive). The majority of tumors exhibit aggressive behavior and high histologic grade; however, they are sensitive to conventional chemotherapy and respond well to anti-Her 2 treatments; additional research to confirm the biomarkers predicting resistance to anti-Her 2 treatment would help ensure the best possible care for patients with this subtype (5).

The College of American Pathologists (CAP) and the American Society of Clinical Oncology (ASCO) state that gene amplification at in situ hybridization (ISH) and/or protein overexpression (score 3+) at immunohistochemistry (IHC) are indicators of a positive HER2 test. However, this paradigm is shifting with the advent of new anti-HER2 drugs since certain breast tumors have lower levels of protein expression (i.e., score 1+/2+ without gene amplification). The possibility of HER2 targeting in estrogen receptor (estrogen receptor)-positive/HER2-negative breast cancers and HER2 "ultra-low" (i.e., score 0 with incomplete and weak staining in ≤10% of tumor cells) has recently come to light (6).

Breast cancer that is triple negative (TNBC) and basal-like (BLBC):

Surrogate IHC detects TNBC when ER, PR, and HER2 responses are negative. At the protein level, it may or may not express cytokeratin (CK 5/6) and/or the Epidermal Growth Factor Receptor (EGFR). As a result, it resembles but differs from BLBC, which GEP has identified. Studies show that there is around 75% concordance between the two entities. Although subsets of low-grade BLBC/TNBC have been identified with a much better prognosis, the majority of TNBCs and BLBCs have morphological

similarities and are biologically aggressive. Although they only cause 10% to 20% of breast cancers, TNBCs and BLBCs are accountable for 30% of breast cancer mortality (5).

A tiny percentage of high-grade TNBCs/BLBCs are metaplastic carcinomas, whereas the majority have NST histology. It's clear that this is still a diverse group with a range of molecular components, and further categorization is necessary for tailored treatment to enhance a patient's prognosis. In recent years, attempts have been undertaken to further categorize TNBC. Lehmann et al. used a 2188-gene set to analyze 21 publicly available data sets with 587 cases of primary BLBC. They discovered that tumors could be further classified into six subtypes (Vanderbilt subtypes) based on their GEP: immunomodulatory (IM), mesenchymal (MES), mesenchymal stemlike (MSL), luminal androgen receptor (LAR), basal-like 1 (BL1), and basal-like 2 (BL2).

When Prat et al. discovered that the gene expression patterns in the IM and MSL groups were from tumor stromal cells rather than cancer cells, they contested the results (7).

The GEP of the LM subtype was shown to be strongly influenced by tumor-infiltrating lymphocytes (TILs) in another investigation by Lehmann et al (8).

Rather than being a distinct subtype, correlation with this signature should be seen as a description of the tumor's immunological status. On the other hand, tumors with a substantial quantity of tumor-associated mesenchymal tissue were included in the MSL subtype. BL1, BL2, MES, and LAR are the four sustainable subtypes that Lehmann et al. developed their categorization into. They claimed that each of these subtypes has unique clinicopathologic characteristics.

Neoadjuvant Chemotherapy for Breast Cancer Treatment

Pathological complete response, or pCR, is defined as Data from neoadjuvant studies was difficult to report and understand in the past due to the lack of a standard criteria for pCR (9). The FDA (Food & Drug Administration) formed a working group called Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC) to address these limitations. The group compared various definitions with long-term outcomes in view of OS and EFS (Event Free Survival) using data from almost 13,000 patients enrolled in large-scale neoadjuvant trials. According to Cortazar et al.(10), there are currently two definitions of pCR that are used when planning studies for U.S. marketing clearance. 1. The absence of residual invasive cancer on hematoxylin and eosin evaluation of the entire resected breast specimen and all sampled regional lymph nodes after neoadjuvant systemic therapy is known as pathological complete response (pCR) (i.e., ypT0/Tis ypN0 in the current AJCC staging system) (10). 2. The absence of residual invasive and in situ cancer on hematoxylin and eosin evaluation of the entire resected breast specimen and all sampled regional lymph nodes after the completion of neoadjuvant systemic therapy is known as pathological complete response (pCR) (i.e., ypT0 ypN0 in the current AJCC staging system) (11).

Pathological full reaction and survival

In order to determine whether chemotherapy (four cycles of doxorubicin and four cycles of cyclophosphamide AC, as was the standard of care at the time) could prolong the DFS and OS in comparison to when administered post-operatively, as well as whether it could increase the rate of breast conservation surgery and node-negative disease, the National Surgical Adjuvant Breast and Bowel Project B-18 (NSABP B-18) designed the landmark study. The reported pCR rate (complete pathological response

and noninvasive disease) was 13% of the 36% complete clinical response. The research found a statistically significant link between initial tumor response and outcome, although no significant difference in OS or DFS was seen between the two groups after 9 years. The OS for patients with pCR was 85%, whereas the DFS and OS for patients with residual tumor were 58% and 73%, respectively. Patients with a pCR had a 50% lower probability of dying than the group after controlling for other prognostic variables (12).

The role of long non-coding RNAs (LncRNAs) in cancer

In 1492, C.H. Waddington used the word "epigenetic" to refer to the way that genes and the environment interact to shape the formation of phenotypes. While epigenetic processes explain how phenotypic changes happen independently of the underlying DNA sequence, genetic mechanisms explain how heritable features result from mutations in the DNA sequence (13).

Because epigenetic modifications create a kind of cellular memory that is transferred to progeny, they guarantee that patterns of differential expression are transmitted steadily when cells divide. Additionally, for select target genes or specific genomic areas, these methods may guarantee the durable inheritance of an activated transcriptional state (13).

On the other hand, they have the ability to reorganize the chromatin of certain genomic areas, resulting in a totally condensed state that is transcriptionally inactive. While epigenetic modifications are reversible and do not alter the DNA sequence, they may alter how the body interprets it, in contrast to genetic modifications. While genetic alterations may change which protein is produced, epigenetic modifications can modify the expression of genes by turning them "on" or "off." Gene expression happens when proteins are transcribed from DNA. Thus, deciphering these epigenetic changes in cancer is essential to figuring out the disease's processes and investigating possible treatment options (14).

Three categories comprise the most important mechanisms for epigenetic labeling: non-coding-mediated RNA modifications (ncRNA), histone modifications (histone methylation, acetylation, phosphorylation, and ubiquitylation), and DNA modifications (DNA methylation and hydroxy-methylation) (13).

First, methylation of DNA: One essential epigenetic alteration that controls gene expression is DNA methylation. A methyl (-CH3) group is added to the DNA cytosine ring throughout the process. About 50% of human genes contain a promoter region rich in C-G sequences, known as CpG sites, in relation to CpG dinucleotides (C-Phosphodiester-G bond) (13). DNA methyltransferase catalyzes DNA methylation, which occurs without changing the genomic DNA sequence. Rather, it influences gene expression by preventing proteins that read the gene from binding. DNA methylation basically makes a gene "OFF." On the other hand, demethylation, which removes the methyl group, makes a gene "ON" (15).

Malignant tumors are linked to aberrant DNA methylation in two primary ways: Gaining methylation in DNA causes hypermethylation, which suppresses transcription and lowers gene expression. Numerous tumor suppressor genes may experience transcriptional suppression due to hypermethylation, which may change signaling pathways and aid in the development of cancer (16).

A lack of methylation is the hallmark of DNA hypomethylation, which may also affect how genes are expressed. Human malignancies may start and spread because hypomethylation events can alter

chromosomal stability and activate oncogenes. Gaining knowledge of DNA methylation dynamics may help one better understand the molecular processes that underlie the initiation and spread of cancer (17).

Modification of histones: The genome is located in the cell nucleus as chromatin, a combination of proteins called histones and DNA. The transcriptional activity is greater in the loosely packed form of DNA, known as euchromatin, and lower in the firmly packed form of DNA, known as heterochromatin (18).

Numerous posttranslational changes may occur in histones, primarily in the terminal tails. Histone tail trimming can influence the recruitment of different factors that impact downstream processes. It is important to note that removing the N-terminal tail of histones also affects chromatin dynamics and structure, which may either increase or decrease transcription activity (19).

Nucleosome dynamics, transcription, and chromatin compaction may all be influenced by epigenetic histone changes and the enzymes that carry them out. By causing gain or loss of function, overexpression, suppression by promoter hypermethylation, chromosomal translocation, mutations of the histone-modifying enzymes/complexes, or even changes to the histone modification site, dysregulation of these processes can upset the balance of gene expression and is thus commonly seen in human cancers (20).

RNAs that do not code: Only over 2% of the genome is known to encode proteins, but over 90% of the genome may be transcribed as non-coding. This non-coding genome is altered in the majority of malignancies. DNA is converted into RNA, which serves as a link between DNA and proteins. Coding RNAs (mRNAs) and non-coding RNAs (ncRNAs) are two types of RNA. Known as oncogenes or tumor suppressor genes, ncRNAs are a varied set of RNA molecules that do not code for proteins but play significant regulatory functions in several cellular processes. Dysregulation of ncRNAs is seen in various cancer types. The kinds of ncRNAs are as follows (21): Small, single-standard RNAs, known as microRNAs (miRNAs), are transcribed from certain genes in the genome and go through a number of processing stages before becoming mature and functional. They are usually 18–25 nucleotides long. Once fully developed, miRNAs have the ability to attach to messenger RNAs (mRNAs) and either stimulate or prevent the translation of mRNAs into proteins. Base-pairing interactions between the miRNA and the target mRNA allow miRNAs to regulate gene expression. Development, cell differentiation, and illness are among the many biological processes in which they are implicated (22).

Essential RNA molecules involved in protein synthesis are known as transfer RNAs (tRNAs). Some tRNAs have been shown to have roles beyond protein synthesis, even though their primary job during translation is transporting amino acids to the ribosome (23).
Short double-stranded RNA molecules known as small interfering RNAs (siRNAs) are essential for controlling genes. They participate in a process known as RNA interference (RNAi) and are typically 20–25 nucleotides long. By attaching to corresponding messenger RNA (mRNA) molecules and blocking their translation into proteins, siRNAs are in charge of silencing certain genes. This method has important ramifications for many biological processes, including as development, illness, and cellular defense systems, and it enables precise regulation of gene expression. Small RNA molecules called piwi-interacting RNAs (piRNAs), which are mostly produced in germline cells and are usually 24-32 nucleotides long, are involved in maintaining genome integrity and silencing transposable elements (23).

Covalently closed RNA molecules that take the shape of a circle are known as circular RNAs, or circRNAs. By interacting with RNA-binding proteins or functioning as microRNA sponges, circRNAs have been shown to control gene expression. Long non-coding RNAs (lncRNAs) are longer RNA molecules that do not encode proteins; they are usually longer than 200 nucleotides. Chromatin remodeling, transcriptional regulation, and post-transcriptional processing are just a few of the ways that long noncoding RNAs (lncRNAs) may control gene expression (22).

Long noncoding RNA localization:

It has been shown that lncRNAs may be found in both the cytoplasm and the nucleus, and they operate in a variety of ways that are all equally significant. The fundamental processes of the majority of lncRNAs are still unknown, however. A significant portion of lncRNAs only have nuclear function. Malignant transformation may result from the deregulation of their expression. Notable processes in the nucleus include interactions with transcription factors, spliceosomes, and epigenetic remodelers, where lncRNAs function as scaffolding, stabilizers, or guides to affect changes in chromatin architecture and gene expression (24).

More precisely, lncRNAs in the nucleus can direct transcription factors and epigenetic remodelers to their target genes or even sequester them to either promote or inhibit the expression of those genes. They can also regulate the ubiquitination and mRNA stability of proteins in the nucleus, which in turn controls their activities. By starting and sustaining chromosomal looping to connect distant enhancer sites and gene promoters, lncRNAs may also modify chromatin in three dimensions (24).

Oncogenesis and LncRNAs

It has been shown that a number of lncRNAs influence the carcinogenesis process; in particular, they often play either an oncogenic or tumor suppressive function in human malignancies. The first discovery of lncRNAs linked to cancer is caused by changes in the genomic sequence, gene dosage, and/or expression levels in tumor cells. Some of the discovered lncRNAs have been directly associated with certain cancer characteristics via subsequent in vitro and in vivo research (25).

Approximately 15% of lncRNAs are up-regulated and 11.18% are down-regulated in seven different types of cancer, including gastric, lung, prostate, breast, pancreatic, hepatocellular, and ovarian cancers. To date, 8,179 lncRNAs have been identified as being essential in the development of at least one type of cancer. Somatic copy number changes (SCNAs) may impact lncRNA dysregulation. Numerous cancer types have been shown to include SCNAs, and a substantial link between RNA expression level and 36.27% of lncRNA gene copy numbers has been established (26).

LncRNAs and resistance to drugs:

In many cancer cells and tissues, including breast, hepatocellular, gastric, colorectal, and cervical cancers, dysregulated lncRNAs contribute to treatment resistance. Numerous processes, such as the inhibition of cell death pathways, the production of excessive drug efflux, the facilitation of autophagy, the modulation of CSC characteristics, and the development of the EMT, contribute to cancer drug resistance. Because of these properties, ncRNAs have a lot of promise as therapeutic agents or targets for the treatment of cancer. The precise treatment of cancer patients, especially those who are not responding well to chemotherapy, may greatly benefit from therapeutic approaches that leverage ncRNAs or directly

target ncRNAs. One intriguing approach to enhance cancer intervention is the transfer of tumor-suppressive ncRNAs to specific cancer cells (5).

For instance, it has been shown that lncRNA UCA1 increases chemoresistance to a variety of drugs in a variety of malignancies, including colorectal cancer (5-FU), prostate cancer (docetaxel), ovarian cancer (cisplatin), chronic myeloid leukemia (imatinib), bladder cancer (cisplatin, gemcitabine), and gastric cancer (MDR). Furthermore, it was shown that overexpressed miR-221 improved cisplatin resistance in osteosarcoma by targeting Protein Phosphatase 2 (PP2A), but it also made pancreatic cancer cells resistant to 5-FU by controlling the gene that codes for Retinoblastoma Protein 1 (RB1) (27).

As a useful tumoral suppressor, LncRNA GAS5 has also been shown to improve treatment efficacy in a number of malignancies, including prostate, pancreatic, and non-small cell lung cancer (NSCLC) (28).

There are a number of lncRNAs that have been shown to express differently in breast cancer than in breast tissues that are not tumorous. For instance, 1758 lncRNAs were deregulated in breast tumors with a triple-negative phenotype when compared to corresponding normal tissues, while over 1300 lncRNAs were discovered to be differently expressed in HER2-positive breast malignancies. These findings highlight the significance of lncRNAs in the emergence of breast cancer. Furthermore, it has been discovered that lncRNAs may be expressed either widely or in a tissue-specific way, and that they can be released into the circulation in a stable form as the illness progresses (4).

Different histological subtypes of breast cancer may also have distinct lncRNA expression patterns. These lncRNAs may be used as therapeutic targets or as biomarkers for diagnosis.

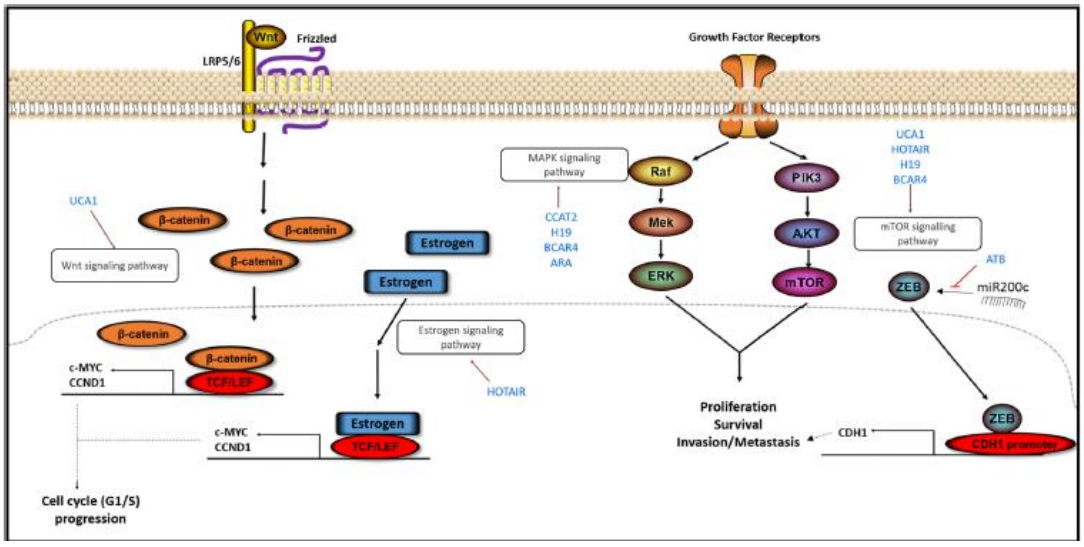

Figure 1: Implications of lncRNA in breast cancer signaling pathways.(Pecero et al., 2019)

H19, HOTAIR, MALAT-1, CCAT1, CCAT2, and other lncRNAs have been shown to have oncogenic roles in breast cancer in recent years. UCA1. GAS5, EPB41L4A-AS2, BC040587, and FGF14-AS2 are lncRNAs that have been shown to have tumor suppressor functions. These lncRNAs have been suggested to function as predictive biomarkers, putative therapeutic targets, and diagnostic/prognostic biomarkers in breast cancer (24).

Response to neoadjuvant chemotherapy and long noncoding RNA in breast cancer

Whether chemotherapy, targeted therapy, or endocrine treatment, a variety of studies have shown the connection between lncRNAs and MDR (multi drug resistance) in BC. Each of these therapies has a unique lncRNA that is in charge of the resistance mechanism. By influencing cell death, triggering the EMT process, and focusing on traditional signaling pathways, the majority of lncRNAs—which are increased in BC cells and tissues—promote MDR (29).

Beyond mRNA signature, an increasing number of lncRNAs have been shown to be linked to patient outcomes and pCR after NACTH, offering a new choice for model building to predict pCR following NACTH. But as of yet, no lncRNA signature has been developed using a large number of BC patients who had NACTH treatment (30).

Although the biological functions of these lncRNAs have not been investigated, a lncRNA signature (LRS) of 36 lncRNAs has been reported to function as a biomarker for predicting pCR following neoadjuvant chemotherapy. Based on this, patients treated with NAC were split into two groups, LRS-high group and LRS-low group, where higher LRS was linked to higher pCR rates (31).

In a different research, Zeng et al. used a lncRNA-mining technique to examine lncRNA expression profile in 1102 BC patients using Gene Expression Omnibus datasets. Analysis was done on the relationship between the lncRNA signature and pathological response (pCR). High expression of lncRNA U79293 was linked to a low chance of pCR, but raised expression of lncRNA AK291479 and BC032585 was linked to a greater risk of pCR (32).

Additionally, Zeng et al. conducted an in vitro investigation to ascertain the susceptibility of cells to the chemotherapeutic drugs Paclitaxel and Doxorubicin. According to Zeng et al. (2019), BC032585 knockdown demonstrated a considerable resistance to chemotherapy and anthracyclines but not paclitaxel in breast cancer cells with or without BC032585 modification. This suggests that BC032585 may be interfering with the pathological response in breast cancer. Additionally, Du et al. found that underexpression of the lncRNA BC0332585 was linked to multidrug resistance, namely to paclitaxel and doxorubicin. By promoting the EMT epithelial mesenchymal transition and activating drug efflux pumps by targeting ABC (ATP Binding Cassette) transporters in BC (32).

A response score comprising a basic gene expression profile of only three RNA species—BPESC 1, WDR72, and GADD45A—one lnCRNA and two coding genes, was successful in predicting pCR for triple negative patients. It was discovered that lncRNAs H19 were trastuzumab responsive in her two positive patients; its expression was elevated in cells that were resistant to trastuzumab, and its downregulation restored the cells' sensitivity to the drug (17).

This opens the door to a more individualized treatment approach with better patient selection for those who will actually benefit from neoadjuvant chemotherapy by offering a new option for model construction to predict pCR after NACTH using lncRNAs in addition to mRNA signature and clinicopathological features. Furthermore, BC drug resistance has been effectively reversed in many experimental settings with the use of anti-miRNAs and miRNA mimics to deplete oncogenic miRNAs and mimic endogenous tumor suppressor miRNAs. Thus, a viable approach to addressing the problem of

medication resistance in BC is the use of specific RNA-based formulations in combination with systemic medicines (33).

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