Review Article

The Role of Long Non-Coding RNAs in Breast Cancer

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Abstract

Long non-coding RNA (lncRNA) genes are an important population of non-coding RNAs with defined key roles in normal development as well as tumorigenesis process. Evidences suggest that they can be classified as tumor suppressor genes or oncogenes according to their functions and expression pattern in tumoral tissues. They have been shown to regulate the plasticity of cancer stem cells. Their important roles in the regulation of cancer-related pathways in addition to deregulation of their expression in a number of cancers have suggested that they can be used as markers for cancer detection and prognosis, as well as targets for cancer treatment. Deregulation of a number of lncRNAs, such as HOTAIR, XIST, MALAT, and H19 has been detected in breast cancer samples and cell lines. In addition, the association between lncRNAs signature and breast cancer patients' survival has been assessed in various studies. Here, the expression patterns of lncRNAs in breast cancer, as well as their significance in prognosis and patient treatment are discussed.

Keywords: Breast cancer, long non-coding RNA, prognosis

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Introduction

Although less than 2% of the human genome encodes protein-coding genes, it has been revealed that at least 90% of the genome is actively transcribed. Long non-coding RNA (lncRNA) genes are an important population of non-coding RNAs, whose critical role in normal development, as well as the tumorigenesis process is being elucidated.[1] It has been estimated that the human genome contains 23,000 lncRNA genes, which are more abundant than 20,000 protein-coding genes.[2] Their physiological and pathological functions have been shown to be exerted via their interactions with microRNAs (miRNAs), mRNAs, proteins and genomic DNA.[3]

The significant increase in the number of lncRNA in genomes of complex organisms indicates their role in the development of such organisms as shown in the processes of dosage compensation, genomic imprinting, cell differentiation and organogenesis.[4]

LncRNAs are actively involved in chromatin rearrangement, histone modification, and modification of alternative splicing genes, as well as regulation of gene expression.[5] They can be intronic, intergenic or in close association with the mRNA genes.[5]

Although lncRNAs share some characteristics with miRNAs, their size, functions and structure are different from them.[6] Table 1 compares miRNA and lncRNA characteristics. The crucial roles of lncRNAs in dosage compensation, imprinting, and homeotic gene expression imply that they can be regarded as part of a network between DNA and definite chromatin remodeling mediators.[7] Some lncRNAs have been shown to silence genes *in trans*, but others function *in cis*.[8] As deregulation of gene expression is an important event in carcinogenesis, it has been suggested that a substantial part of the cancer risk may be attributed to lncRNAs transcribed from cancer-associated loci.[9]

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The role of lncRNA in cancer stem cells (CSCs)

Cancer stem cell (CSC) theory has postulated that tumors originate from a small cell population possessing stem cell properties.[10] Breast cancer has been the first among solid tumors in which the existence of CSCs was documented.[11] Breast CSCs are distinguished by the expression of CD44 but no or low CD24 expression (CD44+CD24-). In addition, aldehyde dehydrogenase 1 (ALDH-1) has been suggested as another possible marker for breast CSCs. The tumorigenic potential of breast CSCs that carry both above-mentioned phenotypes has been shown to be more than CD44+CD24- ones. Furthermore, the expression of ALDH-1 in breast cancer cells has been shown to be associated with estrogen receptor (ER) and progesterone receptor (PR) negative status, while human-epidermal growth factor receptor type 2 (HER-2) positive status, in addition to distant metastases potential and resistance to conventional chemotherapy with paclitaxel and epirubicin.[12] Such cells have been demonstrated to be more prevalent in poorly-differentiated breast cancers compared to well-differentiated ones.[13] In addition to miRNAs, lncRNAs have been shown to regulate the plasticity of CSCs.[3] HOTAIR, XIST, MALAT and H19 are among lncRNAs, whose role in regulation of CSC plasticity has been partly elucidated. Such non-coding RNAs have been suggested as possible targets for anti-CSC treatments.[3] The involvement of HOX genes in CSCs pathways,[14] in addition to the observed roles of some lncRNAs in the regulation of HOX gene expression, provide further evidences for lncRNAs participation in tumorigenesis. SOX2OT is a recently identified lncRNA, which has been shown to induce the expression of SOX2, an essential factor for maintenance of pluripotency in breast cancer stem cells.[15] In addition, the role of lncRNAs in the epithelial-to-mesenchymal transition (EMT) programs has been partly elucidated. Such programs have been shown to be involved in cancer progression and metastasis in addition to the acquirement of stem cell characteristics.[16] Hundreds of lncRNAs have been demonstrated to be up-regulated and down-regulated during the EMT. Among them, lncRNA-HIT (HOXA transcript induced by TGF-β) has been shown to mediate TGF-β function

Table 1. Comparison of miRNAs and lncRNAs.

Features	miRNAs	lncRNAs
Size	21– 24 nt	≥ 200 nt
Transcription	Mostly RNA Pol II (In some cases RNA Pol III)	Mostly RNA Pol II (In some cases RNA Pol III)
5' capping	Yes	Yes
Poly Adenylation	Yes	Yes
Splicing	Yes	Yes
Translation	No	Rarely produce some peptides
Location	Exonic (Sense/Antisense) Intronic Intergenic	Exonic (Sense/Antisense) Intronic Intergenic Bidirectional Overlapping
Conservation	High	Low
Tissue specificity	Low	High
Function	Post-transcriptional regulation mRNA Decay (destabilization) mRNA Cleavage Cap-40S initiation inhibition 60S Ribosomal unit joining inhibition Elongation inhibition Ribosome drop-off (premature termination) Co-translational nascent protein degradation Sequestration in P-bodies	Regulation of transcription Regulation of gene-specific transcription Regulation of basal transcription machinery Post-transcriptional regulation splicing translation Sirna-directed gene regulation Epigenetic regulation Chromatin remodeling Imprinting X-chromosome inactivation Telomeric non-coding RNAs
Interactions among miRNAs & lncRNAs	Trigger lncRNAs decay Compete with lncRNAs for interaction with mRNAs	Sponge/decoy miRNAs Generate miRNAs Compete with miRNAs for interaction with mRNAs

in cell migration, invasion, tumor growth, and metastasis.[17] ATB is another lncRNA, whose high level of expression in human has been shown to be correlated with trastuzumab resistance of breast cancer patients and is suggested to be a mediator of TGF-β signaling predisposing breast cancer patients to EMT.[18] Besides, several lncRNAs are involved in regulation or activation of WNT signaling pathway in the Twist-induced EMT process.[19]

In brief, the role of lncRNAs in self-renewal of embryonic and pluripotent stem cells is evident, but their involvement in the transformation process of such cells or therapeutic resistance of CSCs need to be clarified.[20]

The role of lncRNAs in breast cancer evolution

LncRNAs have been shown to be involved in mammary gland development, as well as breast cancer evolution.[21] For instance, PINC (pregnancy-induced non-coding RNA) is involved in cell survival and cell cycle progression and has been shown to inhibit mammary cell differentiation.[22] Many lncRNAs have been shown to be aberrantly expressed in breast cancer. According to their functions and pattern of expression, they can be divided to tumor suppressors and oncogene classes. Table 2 shows the lncRNAs involved in breast cancer with more clinically relevant ones described in the text.[23–47] Based on the rapid pace of lncRNA discovery in recent years (Figure 1), it is expected that such list will contain more lncRNAs in future. LncRNAs have been suggested as molecular markers for characterization of ductal carcinoma in situ (DCIS) subtypes. Such classification may be useful for prediction of progression from in situ to invasive cancer.[48] In addition, a number of lncRNAs have been shown to predict patients' survival. For instance, a set of four lncRNA genes (U79277, AK024118, BC040204 and AK000974) have been recognized, using the lncRNA-mining approach, which is predictive of breast cancer patient survival. It has been shown that this lncRNA expression signature is independent of age and cancer subtype. This gene set is shown to be implicated in numerous cancer metastasis related pathways.[49] A recent study has identified 3 lncRNA genes (LINC00324, PTPRGAS1 and SNHG17), whose expression pattern is associated with ER^+ and ER^- subtypes, tumor histologic grade, as well as clinical outcomes.[36]

Antisense lncRNAs

ANRASSF1 (RASSF1 antisense RNA 1)

It is an endogenous un-spliced lncRNA that is transcribed from the opposite strand on the RASSF1 tumor suppressor gene. Its expression has been shown to be higher in breast and prostate tumor cell lines compared with non-tumor cells, while RASSF1A has an opposite pattern. Ectopic overexpression of this lncRNA in HeLa cells has resulted in diminished RASSF1A expression while an increase in the proliferation of these cells.[25]

ANRIL (Antisense non-coding RNA in the INK4 locus)

Its involvement in tumorigenesis processes has been shown in several cancers and it is thought to be exerted through regulation of its adjacent tumor suppressors CDKN2A/B via epigenetic mechanisms.[50] It has a number of splicing variants with most of them being polyadenylated. The tissue specificity of some of the splicing variants has suggested their physiological significance in the certain tissues.[51] ANRIL has been shown to be involved in ATM-dependent DNA damage response, as it has been upregulated

Table 2. List of lncRNAs involved in breast cancer.

LncRNA	Chromosomal location	Alteration in breast cancer	Position relative to coding genes	Involvement in other cancers	Functions/ characteristics	Level of applied assays	References
AC005152.3	17q24.3	upregulation	intergenic	- - -	Overexpressed in basal like breast cancer subtype	Human breast cancer tissue	23
Alpha-HIF	14q23.2	upregulation	overlapping, antisense	Renal	Regulates HIF-1a mRNA A marker of poor prognosis in breast cancer	Human breast cancer tissue	24
ANRASSF1	3p21.3	upregulation	overlapping, antisense	- - -	Decreases the expression of RASSF1 tumor suppressor gene	Breast cell lines (MCF7, MCF10A and MBA-MB-231), Prostate cell lines (RWPE-1, LNCaP and DU145)	25
ANRIL	9p21.3	N/A	overlapping, antisense	Esophageal, Gastric, Leukemia, Melanoma, Neurofibromatosis type 1, Prostate, Basal cell Carcinoma, Glioma	Regulate its neighbor tumor suppressors CDKN2A/B by epigenetic mechanisms & regulate cell proliferation	Fibroblast cell line (GM0637), Osteosarcoma cell line (U2OS), HCT116 $p53^{+/+}$ and HCT116 p53–/– cell lines	26
ARA (LOC104968398)	Xq23	upregulation	intronic	- - -	Modulate MAPK signaling pathway, metabolism pathways, cell cycle and cell adhesion-related pathways	Breast cancer cell line (MCF-7), Liver cell line (HepG2)	27
ATB	14q11.2	upregulation	intergenic	Hepatocellular carcinoma	Has role in epithelial to mesenchymal transition	Human breast cancer tissue, Breast cancer cell line (SKBR-3)	18
BC040587	3q13.31	downregulation	intergenic	Osteosarcoma	An independent prognostic biomarker in breast cancer	Human breast cancer tissue	28
BCAR4	16p13.13	upregulation	intergenic	- - -	Induces antioestrogen resistance but sensitizes breast cancer to lapatinib	Human breast tissue, Breast cancer cell lines (MCF-7 and ZR-75-1)	29
BCYRN1 (BC200)	2p21	upregulation	intronic	Cervical, Lung, Esophageal, Ovarian, Parotid, Tongue	A molecular indicator of invasive malignancy	Human breast cancer tissue	21
CCAT2	8q24.21	upregulation	intronic	Non-small cell lung cancer	Upregulates cell migration and downregulates chemosensitivity to 5'FU	Human breast cancer tissue Cell lines (CAMA1, MCF7, ZR75, BT474, SKBR3, MM231, MM435, COLO320, DLD1, HT29, HCT116)	30
DSCAM-AS1	21q22.2	upregulation	intronic	- - -	Upregulated during the malignant progression of carcinomas by an oestrogen-indipendent mechanism	Human breast cancer tissue	31
GAS5	1q25.1	downregulation	overlapping	Kidney, Lymphoma, Prostate	Induces growth arrest and apoptosis Overexpression of some GAS5 transcripts has lead to growth arrest and apoptosis in breast cancer cell lines	Human breast cancer tissue, Breast cell lines (MCF-7, MDA-MB-231, MCF10A, Hs578T and T47D)	32
H19	11p15.5	upregulation	overlapping	Bladder, Cervical, Colon, Esophageal, Gastric, Glioblastoma, Hepatocellular, Lung, Ovarian, Prostate, Melanoma, Meningioma, Adrenocortical carcinoma	Its downregulation decreases breast cancer cell clonogenicity & anchorage-independent growth.	Human breast cancer tissue, Breast cell lines (MCF-7, MDA-MB-231)	33
HIT	N/A	upregulation	N/A	- - -	Identified in mice breast cancer cell line Has a role in epithelial to mesenchymal transition	Animal model (Mus musculus 4T1), Mouse mammary gland cell line (NMuMG)	17

HOTAIR	12q13.13	upregulation	overlapping, antisense	Colorectal, Cervical, Endometrial, Gastric, Squamous cell, Gastrointestinal, Hepatocellular, Liver, Lung, Pancreas, Small cell lung cancer	Loss of HOTAIR can inhibit cancer invasiveness especially in cells that possess excessive PRC2 activity.	Human breast cancer tissue	34
HOTAIRM1	7p15.2	upregulation	antisense	Leukemia	Its expression is correlated with the expression of the HOXA1.	Human breast cancer tissue	23
IRAIN	15q26.3	N/A	overlapping	- - -	- - -	Human breast cancer tissue Breast cell lines (MCF-7, MDA-MB-231)	35
LINC00324	17p 13.1	N/A	intergenic	- - -	Differentially expressed between ER^+ & ER^- subtypes	Human breast cancer tissue	36
LINC00472	6q13	downregulation	intergenic	- - -	LINC00472 expression could suppress breast cancer cell proliferation and migration.	Human breast cancer tissue, Breast cell lines (MCF-7 and SKBR3)	37
LSINCT5	5p15.33	upregulation	N/A	Ovarian	LSINCT5 knocking down in cancer-derived cell lines causes a decrease in cellular proliferation.	Breast cell lines (MCF-7, MCF10A, T47D, HCC1500)	38
MALAT1	11q 13.1	upregulation	intergenic	Bladder, Cervical, Endometrial, Colorectal, Hepatocellular, Kidney, Liver, Lung, Neuroblastoma, Non-small cell lung cancer, Osteosarcoma, Pancreas, Prostate, Uterus	Plays a critical role in pre-mRNA alternative splicing	Human breast cancer tissue	39
MEG3	14q 32.2	downregulation	intergenic	AML, Bladder, CML, Colon, Gastric, Glioma, Hepatocellular, Kidney, Lung, Meningioma, Neuroblastoma, Prostate	Regulates the TGF-β pathway genes through formation of RNA–DNA triplex structures	Breast cell line (BT549)	40
MIR31HG	9p 21.3	upregulation	overlapping	- - -	miR-31 play a role in the invasion-metastasis cascade of breast tumors & transcribed from within the first intron of MIR31HG.	Breast cell line (MCF10A)	41
PTPRG-AS1	3p14.2	N/A	overlapping, antisense	- - -	Differentially expressed between ER^+ & ER^- subtypes	Human breast cancer tissue	36
PVT1	8q24	upregulation	intergenic	Burkitt, Hodgkins lymphoma, Ovarian, Pancreas, Prostate, Renal	Silencing of PVT1 expression decreases cell proliferation & increases apoptosis in breast cancer cell lines. PVT1 is a MYC activator.	Breast cell line (MDA-MB-231)	42
SNHG17	20q11.23	N/A	overlapping	- - -	Differentially expressed between ER^+ & ER^- subtypes. Its expression in breast cancer correlates with tumor grade. Low expression of it has been associated with the long survival times of breast cancer patients.	Human breast cancer tissue	36
SOX2OT	3q26.33	upregulation	overlapping	Lung cancer	Plays a key role in the induction and/or maintenance of SOX2 expression.	Animal model (Mus musculus) Breast cell lines (MCF-7, MCF10A)	15

SPRY4-IT1	5q31.3	upregulation	intronic	Renal, Melanoma, Bladder, Gastric, NSCLC	A novel prognostic biomarker and a potential therapeutic candidate for breast cancer.	Human breast cancer tissue Breast cell line (SKBR-3)	18
SRA1	5q31	upregulation	intergenic	Ovarian, Uterus	Transcriptional co-activator of steroid hormone receptors	Breast cell lines	43
TRERNA	20q13.13	upregulation	intergenic	- - -	Downregulates the expression of epithelial marker E-cadherin. Enhances invasion, metastasis and possibly epithelial-mesenchymal transition	Animal model (Mus musculus), Cell lines (MCF-7, HEK293T cell)	44
UCA1	19p13.12	upregulation	intergenic	Bladder, Oral Squamous cell carcinoma	The interaction of UCA1 with hnRNP I suppresses the p27 protein level by competitive inhibition.	Animal model (Mus musculus), Breast cell lines (MCF-7, MDA-MB-231, HCT-116 p53-WT and HCT-116 p53-null cells)	45
XIST	Xq13.2	downregulation	intergenic	Bladder, Testicular, Female cancers	Loss of BRCA1 function can lead to loss of XIST RNA association with the inactivated X.	Human breast cancer tissue, Breast cell line (MCF-7)	46
ZFAS1	20q13.13	downregulation	overlapping, antisense	- - -	Knock down of Zfas1 in mouse mammary epithelial cells resulted in a significant increase in proliferation and metabolic activity.	Animal model (Mus musculus), Mouse and human cell lines (HC11, T47D, BT474, MCF7 and N2A)	47

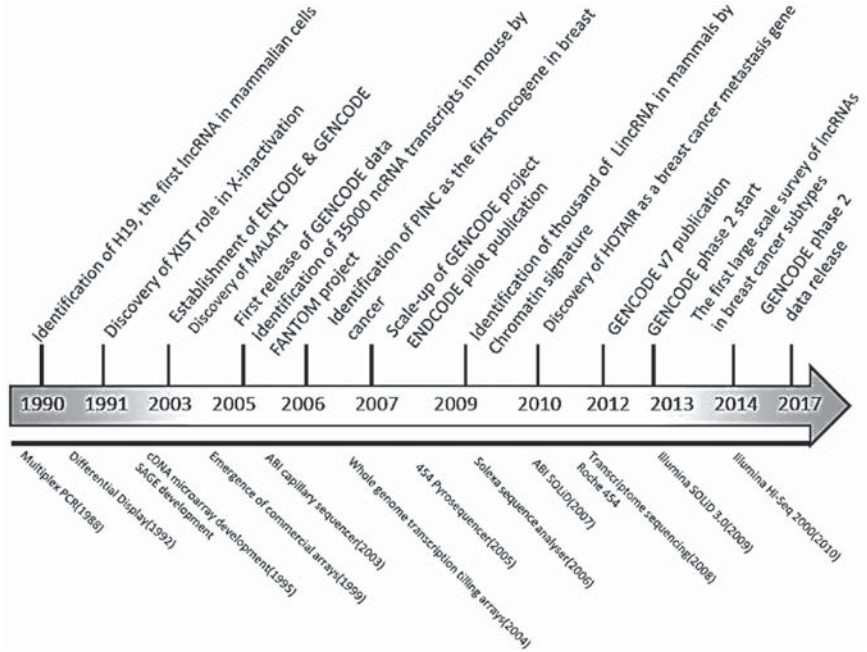

Figure 1. Advances in discovery of lncRNAs with a focus on breast cancer (modified and updated from Gibb, Brown and Lam 2011).

after DNA damage by the transcription factor E2F1. In addition, increased levels of ANRIL inhibit the expression of INK4a, INK4b and ARF at the late-stage of DNA damage response.[26]

HOTAIR (HOX transcript antisense RNA)

HOTAIR is an lncRNA, which is transcribed from HOXC locus and inhibits transcription *in trans* across long distance of the HOXD locus.[52] It has been shown to repress the expression of a number of tumor suppressor genes, namely the protocadherin family, such as HOXD10 and PGR, in addition to various metastasis suppressor genes such as PCDH10, PCDHB5, and JAM2.[53] HOTAIR overexpression has been demonstrated in primary breast tumors and metastases. Its expression level in primary tumors is predictive of eventual metastasis and death. Its upregulation has been shown to alter gene expression pattern of breast epithelial cells to a pattern similar to embryonic and neonatal fibroblasts, whereas its downregulation has changed pattern of H3K27 methylation and decreased invasiveness.[54] Another study has shown that its expression may be an independent biomarker for the prediction of metastasis risk in ER^+ breast cancer patients.[55] Furthermore, its transcription has been shown to be induced by estrogen while repressed by tamoxifen.[56] A recent study has shown its significant overexpression in the HER2-enriched breast cancer subgroup.[23]

HOTAIRM1 (HOXA transcript antisense RNA, myeloid-specific 1)

HOTAIRM1 is a newly discovered lncRNA, which has been shown to interact with polycomb repressive complexes 1 (PRC1) and 2 (PRC2). HOTAIRM1 expression has been demonstrated to be positively correlated with the expression of the HOXA1 neighboring gene. In addition, it has been shown to be significantly overexpressed in the basal-like breast cancer subgroup.[23]

PTPRG-AS1 (Protein tyrosine phosphatase, receptor type, G, antisense)

It is an antisense lncRNA complementary to PTPRG tumor suppressor gene in breast cancer. PTPRG is a member of the protein tyrosine phosphatase family. This family participates in regulation of cell growth and mitotic cycle. A recent study has shown that downregulation of PTPRG-AS1 in human breast cancer tissues is associated with longer patient survival.[36]

Intronic lncRNAs

ARA (Adriamycin resistance associated)

Intronic lncRNAs have been shown to be originated from an intron of PAK3 gene. Its expression in breast cancer cell line is notably associated with adriamycin sensitivity and is significantly upregulated in cell lines following adriamycin treatment. Its knockdown has resulted in reduced cell proliferation, increased cell death, G2/M arrest, and migration defects. In addition, its ability to modulate various signaling pathways, such as a MAPK signaling pathway, metabolism pathways, cell cycle and cell adhesion-related pathways have been shown by microarray transcriptomic analysis.[27]

BCYRN1 (BC200 or Brain Cytoplasmic RNA 1)

BCYRN1 gene encodes a neural small cytoplasmic RNA and has been shown to be one of the few transcriptionally active Alu sequences.[57] It has been shown to act as a neuron-specific translational modulator involved in the regulation of local synaptodendritic protein synthesis in neurons. Its expression has been detected in a number of human tumors, including invasive breast carcinomas with no significant expression in normal breast tissue or in benign tumors such as fibroadenomas. Its expression in DCIS has been suggested as a prognostic marker for tumor progression. Consequently, it has been suggested as a molecular tool in the diagnosis and/or prognosis of breast cancer.[58]

CCAT2 (Colon cancer associated transcript 2)

CCAT2 is located in chromosome 8q24.21 near to numerous mutational hot-spots and has been associated with diverse cancers, including colorectal, prostate, breast and chronic lymphocytic leukemia.[59] One important transcription factor located in its vicinity is transcription factor c-Myc, which is a key regulator of cell cycle, proliferation, differentiation, and apoptosis. CCAT2 has been shown to increase cell migration in human breast cancer cells and decrease chemosensitivity to 5'FU.[30] Although the mechanism of its deregulation in breast cancer cells is not clarified yet, studies in gastric and colon carcinomas have demonstrated its direct induction by c-Myc.[60]

Overlapping lncRNAs

H19

H19 is an imprinted gene with maternal monoallelic expression in fetal tissues, while down regulation in most tissues after birth. It lies in a region of imprinted cluster on 11p15.5, which has been involved in a number of pediatric and adult tumors.[61] H19 has been shown to be the precursor of mir-675.[62] Its expression has been shown to be repressed by p53 protein.[63] Ectopic overexpression of H19 gene has been shown to increase the tumorigenic properties of breast cancer cells when injected into severe combined immunodeficiency (SCID) mice.[64] Its active association with E2F1 to enhance cell cycle progression in breast cancer cells provides further support for its oncogenic role in breast tumorigenesis[65]. In addition, the known oncogene, c-Myc, has been shown to notably upregulate H19 expression in various cell types, including breast epithelial. C-Myc and H19 expressions in human breast carcinomas have been shown to be significantly associated with each other. Furthermore, downregulation of H19 in breast cancer cells reduces cancer cell clonogenicity and anchorage-independent growth supporting an important function for H19 in transformation.[66] Recently, it has been shown that H19 has a role in promotion of tumor metastasis through miR-675 and its expression level considerably associates with the metastatic potential of breast cancer cells.[67] The important network constructed between H19 and tumor suppressor genes, as well as oncogenes provides clues for crucial role of H19 in tumorigenesis.

SOX2OT (SOX2 overlapping transcript)

SOX2, an important transcription factor and developmental gene, has been shown to lie in an intron of this lncRNA gene. The expression of these two genes has been demonstrated to be concordant in breast cancer with higher expressions in ER^+ than in ER^- samples. In addition, suspension culture conditions that support the growth of stem cell phenotypes could upregulate the expression of both genes. Ectopic expression of SOX2OT in MDA-MB-231 cells has resulted in a significant increase in SOX2 expression, accompanied by a diminished proliferation and an increased anchorage-independent growth in such cells. As SOX2 has a crucial role in maintaining pluripotency in an array of stem cells and is involved in CSC maintenance, the role of SOX2OT

in regulation of its expression and its consequent function in tumorigenesis process is important.[15]

Intergenic lncRNAs

LINC00324 (Long intergenic non-coding 00324)

LINC00324 is located in downstream of CTC1 gene. CTC1 is involved in DNA replication and protection of telomeres from degradation. LINC00324 is among lncRNAs, whose expression in human breast cancer is associated with ER status and tumor grade. In addition, its high expression in breast cancer patients has been shown to be correlated with the longer survival of patients.[36]

LSINCT5 (Long stress induced non-coding transcript 5)

LSINCT transcripts have been shown to be overexpressed in the $HER2^-$ and $TP53^+$ breast cell lines.[68] LSINCT5 is a member of this family, whose expression has been shown to be upregulated in breast and ovarian cancers compared to the normal counterparts. In addition, it has been shown to increase cellular proliferation. Its knock down has resulted in significant changes in the expression of a number of proliferations and cancer associated genes, among them are lncRNA NEAT-1 and a protein-coding gene PSPC1, which have been downregulated.[38]

MALAT1 (Metastasis-associated lung Adenocarcinoma transcript-1)

MALAT1 has been shown to modulate alternative splicing of pre-mRNAs.[69] Its participation in tumorigenesis processes, as well as metastasis has been shown in patients with lung cancer.[70] Another study has shown that it regulates gene expression but not alternative splicing in lung metastases.[71] Its abundant expression has been detected in primary breast tumors. In addition, MALAT1 gene mutations and deletions have been detected in luminal breast cancer, especially in the region that could mediate its interaction with a splicing factor named SRSF1.[39]

SRA1 (Steroid Receptor RNA Activator 1)

Although SRA1 has been initially identified as a non-coding RNA gene, it can be alternatively transcribed to a protein coding RNA with some similar functions to the non-coding transcript.[72] It functions as a nuclear coactivator of some steroid hormone receptors, including estrogen and progesterone,[73] as well as some non-steroid nuclear receptors and other transcription factors.[72] It has been shown to regulate cell death in a transgenic mouse model. However, its overexpression is not sufficient to provoke tumorigenesis.[74] In addition, it has been demonstrated that the balance between coding and non-coding SRA transcripts is important in modulating the action of ER and PR, as well as tumor phenotype characterization and is probably involved in breast tumorigenesis and tumor progression.[75] This has been evidenced by significant higher expression of SRA non-coding RNAs in invasive breast cancer cells than non-invasive cells, as well as the lowest relative level of non-coding SRA RNA in normal breast cell line.[76] Therefore, SRA may participate in hormonal carcinogenesis and response to ER targeting treatment strategies, which has been supported by decreased invasiveness of cells following small interfering RNA (siRNA) mediated SRA knockdown.[77]

XIST (X inactive specific transcript)

XIST is a non-coding RNA, which takes part in the beginning of X chromosome inactivation during early embryogenesis. Its expression has been demonstrated to be dysregulated in numerous human cancers accompanied by the absence of inactivated X chromosome (Xi) while duplication of active X chromosome.[78] This phenomenon is more prominent in some cancers, including breast cancer. The famous breast cancer susceptibility gene BRCA1 has been shown to localize to the Xi, interact with XIST RNA and participate in the correct Xi heterochromatin superstructure.[79] Additionally, loss of Xi has been demonstrated in highly aggressive, sporadic basal-like human breast cancers, suggesting a role for X chromosome abnormalities in the pathogenesis of both inherited and sporadic breast cancers.[80]

Development of lncRNA-based cancer therapies

The vast deregulation of lncRNAs in cancers, as well as their involvement in various cancer related pathways implies that they can be used as specific targets for cancer treatment. According to their role in cancer development, different lncRNAs-based therapies can be designed. First strategy would be the reintroduction of wild-type lncRNA with the tumor suppressor function to cells lacking it, which can be done with different delivery systems. Alternatively, antisense oligonucleotides (ASOs), siRNAs, as well as viral vectors that contain short hairpin RNA (shRNAs) can be used for modification of oncogenic lncRNAs expression. The result of ASO-mediated inhibition of lncRNAs in animal models has been promising. A novel modified ASO technology is antagoNAT, which lead to mRNA de-repression via prevention of the interactions of natural antisense transcripts (NATs) with effector proteins or by induction of RNAase H-mediated degradation of the antisense transcript.[81] Such *in vivo* endogenous gene, upregulation has been successful in increasing the level of BDNF protein in neuronal cells via decreasing its antisense.[82] The same strategy can be used for downregulation of lncRNAs, which act as antisense of tumor suppressor genes.

A recent study has shown the effect of ASO-mediated suppression of MALAT1 in the prevention of tumor metastasis in a mouse lung cancer xenograft model.[71] Another study has indicated that ASO-mediated silencing of an lncRNA named CCAT1-L can result in down regulation of the MYC oncogene in different colorectal cancer cells.[83]

SiRNA-mediated silencing of oncogenic lncRNAs seems to be practical in inhibition of cancer cell proliferation and metastasis ability. For instance, siRNA-mediated knockdown of PRNCR1 has resulted in a decrease in the viability of prostate cancer cells.[84] In addition, inhibition of HOTAIR could decrease invasiveness and reverse EMT process in breast cancer cells.[54] Furthermore, SRA1 knock down in breast cancer cell line decreased their invasiveness and expression of genes related to this process.[77] Additionally, inhibition of a novel lncRNA (LINC01212), which is specifically up regulated in melanoma (but not other tumor) cells, as compared to melanocytes, has been shown to induce apoptosis and proposed as a new therapeutic approach in the treatment of melanoma.[85] Such studies highlight the potential of these lncRNAs as targets for cancer therapy. However, it has been revealed that siRNA knockdown strategy is more difficult in lncRNAs silencing compared to mRNAs, probably because of extensive secondary structures in lincRNAs.[8]

The results of above-mentioned experiments would pave the way toward introduction of clinical trials. Approximately, 100 ASOs and 40 RNA interference-based therapies are being tested in clinical trials in very recent years. More than 20 of these clinical trials have passed the initial phases.[86]

Another therapeutic strategy would be the modification of splicing events to produce a certain splicing variant. Modification of SRA coding and non-coding variants has been suggested as a therapeutic tool in breast cancer.[75] Alternatively, inhibition of interaction of lncRNAs with their targets can be applied as a therapeutic modality. The effectiveness of this modality has been verified in a study which has shown that inhibition of HOTAIR interactions with the PRC2 or LSD1 complexes can decrease the metastatic potential of breast cancer cells.[8] Although not applied in breast cancer patients yet, a plasmid (BC-819) carrying diphtheria toxin under the control of the H19 regulatory sequence has been injected in tumors with H19 overexpression to decrease tumor size.[87]

In conclusion, although mRNA and miRNAs expressions have been suggested as biomarkers for early detection and therapeutic response monitoring in cancer patients for a relatively long time,[88] lncRNAs investigations in cancer are in their infancy. However, numerous lncRNAs are being discovered which can be used as tumor biomarkers.[89] The more cell type specificity of lncRNAs than protein-coding genes facilitate such application.[9] They have been shown to participate in complex gene-environment interactions leading to cancer. With the use of new technologies such as microarray and large-scale transcriptome sequencing techniques, the number of such lncRNAs is increasing. Global sequencing of RNA populations is a novel method for evaluation of lncRNA expression in tumors, which is superior to microarray because lncRNA specific probes are underrepresented on commercial microarrays.[2] Many of lncRNAs identified by such methods have been suggested as markers for cancer diagnosis or prognosis. In addition, they have been shown to participate in modulating the cancer epigenome.[23] In addition to their possible application in treatment, lncRNAs can be used for classification of cancer subtypes. Numerous researches have provided evidences suggesting that expression analysis of mRNA genes is a valuable tool for classification of breast cancer, as well as evaluation of survival and prognosis.[90,91] LncRNAs expression analysis is also expected to facilitate such evaluations especially in situations that the expression analysis of coding genes seems to be inadequate for prediction of response to a certain treatment. An example of such situation is the relative resistance of some ER^+ breast cancer patients to tamoxifen, which can be partly explained by differential expression of lncRNAs involved in estrogen signaling pathways. A recent study has indicated that ER expression is associated with distinctive lncRNA networks.[23] Consequently; lncRNAs provide a vast field for researchers to identify cancer-related pathways, as well as designing novel treatment modalities.

Author's Contribution

Mohammad Soudyab and Mostafa Iranpour equally contributed as first authors.

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