Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

HHS Public Access
Author manuscript
Front Biosci (Schol Ed). Author manuscript; available in PMC 2017 October 23.

Published in final edited form as:
Front Biosci (Schol Ed). ; 7: 94–108.

Roles of lncRNA in breast cancer

Yin Liu[1], **Sambad Sharma**[1], and **Kounosuke Watabe**[1,*]

[1]Department of Cancer Biology and Comprehensive Cancer Center, Wake Forest University School of Medicine, Winston-Salem, North Carolina, USA

Abstract

Recent systematic genomic studies have revealed a broad spectrum of lncRNAs that are involved in a variety of diseases, including tumor progression, by regulating gene expression at epigenetic, transcriptional and post-transcriptional levels. However, their exact mechanisms of action are yet to be clarified. In breast cancer research, several lncRNAs are identified as tumor driving oncogenic lncRNAs and few are identified as tumor suppressive lncRNAs. They are involved in cell growth, apoptosis, cell migration and invasiveness as well as cancer cell stemness. Therefore, this new class of RNAs may serve as biomarkers for diagnostic and prognostic purpose and also as potential therapeutic targets. This review summarizes the current information about lncRNAs that are particularly involved in breast cancer progression and also discusses the potential translational application of these newly discovered nucleic acids.

1. Introduction

Recent genomic and bioinformatic studies across the species have revealed that eukaryotic genomes transcribe broad spectrum of RNAs including protein coding mRNAs, short non-coding transcripts and long non-coding RNAs (lncRNAs) (1). Among these RNAs, non-coding transcripts are found to be more abundant in human cells. Up to 70% of the human genome is transcribed; however, only 2% of them are translated into protein (2). In the past several years, short non-coding RNAs such as microRNAs, small interfering RNAs and snoRNAs have been extensively studied, while lncRNAs have drawn relatively less attention. However, it has become increasingly apparent that lncRNAs are not simply leaky products of the genome, and many of these RNAs have been experimentally characterized to show their distinct cellular functions. Importantly, many of these transcripts are associated with a variety of human diseases.

LncRNAs can be defined as RNA transcripts longer than 200bp that lack open reading frames. A number of lncRNAs were initially identified through the whole genome tilling array and the next generation sequencing of transcriptome. These studies showed that lncRNAs have complicated structures and intrinsic origins, and therefore, they can no longer be defined just by their length and protein-coding incapability. However, lncRNAs have several common features. The chromatin state of lncRNAs are consistent with protein coding genes, marked by trimethylation of lysine 4 of histone H3 (H3K4me3) at gene

[*]Corresponding author: Kounosuke Watabe Ph.D. Department of Cancer Biology and Comprehensive Cancer Center, Wake Forest University School of Medicine, Winston-Salem, North Carolina, Phone: 336-716-0231, kwatabe@wakehealth.edu.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

promoter and trimethylation of lysine 36 of histone H3 (H3K36me3) along the transcribed region (3). Expression of lncRNAs are often regulated by well-known transcription factors, and the ENCODE project reported that there are multiple transcription factors that preferentially regulate lncRNA transcription (4). Like the coding genes, lncRNAs are transcribed by RNA polymerase II and usually spliced by the spliceosome. LncRNAs also have a polyA tail as the coding mRNAs (3).

LncRNAs can be defined into five categories depending on their origin in the genome as shown in Figure 1 (5). The first group is **Sense lncRNA.** Sequence of these lncRNAs overlap with one or more exons of another transcript in the same direction. The second category is **Antisense lncRNA.** Sequence of these lncRNAs overlap with one or more exons of another transcript in the opposite direction. The third category is **Bidirectional lncRNA, and** these lncRNAs are initiated in close genomic proximity with a neighboring protein coding transcript on the opposite strand. The fourth group is **Intronic lncRNAs.** These lncRNAs are wholly derived from within an intron of another transcript. Sometimes these may represent pre-mRNA sequence. The last category is **Intergenic lncRNAs**, and these lncRNAs are derived from sequence within the genomic interval between two genes.

1.1 Physiological functions of LncRNAs

Recent comprehensive study of the human genome have identified over 8,000 lncRNAs (5). Other studies also reported that there are several thousands of lncRNAs expressed in human and other mammals (3, 6). However, many of these transcripts are not conserved in closely related species, and only ~200 lncRNAs are functionally characterized and mechanistically well defined (7). This raises the question that whether all lncRNAs are biochemically functional. Most lncRNAs are expressed in a tissue specific manner (5), suggesting the potential biological and physiological functions. Among the 200 lncRNAs that have been studied so far, many of them showed evidence of functionality in vitro or in vivo, although only a few were characterized *in vivo*. Judging from the data to date, it is clear that lncRNAs are involved in a wide range of biological and physiological processes and hold distinct functions at each step. Moreover, lncRNAs are important regulators in tumor-suppressor and oncogenic pathways, and recent studies showed that lncRNAs regulate the major cancer-driving pathways at epigenetic, transcriptional and post-transcriptional level as outlined below (3).

1.1.1 Epigenetic regulation—The expression of genes are often controlled by epigenetic factors such as chromatin-modifying complexes and DNA methyltransferases (8), and many tumor suppressive genes are found to be inactivated by epigenetic silencing during tumor progression (9). The most well-known function of lncRNAs is related to epigenetic regulation of target genes, especially by their repressive functions. Many lncRNAs, including ANRIL, HOTAIR, H19 and XIST, achieve transcription repression by coupling with histone-modifying or chromatin-remodeling proteins (10). LncRNAs are believed to be operated in both cis and trans dependent manners, although this concept is still controversial. The cis-acting lncRNAs are restricted to the site or chromosome of synthesis and tend to regulate only a few genes, while the trans-acting lncRNAs diffuse to other chromosomes and regulate wider range of genes (11).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Polycomb respressive complexes (PRCs) are the most common protein partners of lncRNAs that are studied so far. PRCs are known to promote gene repression by modifying the chromatin structure. Intrinsic histone methyltransferases activity maintains gene repression by methylation of specific amino acid on histone tails that result in compaction of chromatin and formation of heterochromatin (12). Several large lncRNAs are known to interact with PRCs (13). One study in 2009 indicated that more than 20% of the lncRNAs interact with PRCs (6). LncRNA transcripts are critical for recruitment of the proteins in some cases where lncRNAs serve as scaffold for protein complexes. HOTAIR is known to scaffold PRC2 and LSD1 that localize to thousands of sites throughout the genome (14).

1.1.2 Transcriptional regulation—Several lines of evidence suggest that lncRNAs directly regulate gene expression by influencing the activity of promoter enhancers. Kim *et al.* recently found that more than 12,000 neuronal activity-regulated enhancers are transcribed bi-directionally to a class of lncRNA called enhancer RNAs (eRNAs) and that the expression of eRNAs was positively correlated to the mRNA level of nearby protein coding genes (15). Other groups also observed noncoding transcripts expressed from enhancer sites nearby the protein coding genes (16, 17). The biological and mechanistic roles of eRNA are not clearly defined yet, although a few were functionally studied (17). Androgen receptor (AR) is a type of nuclear receptor that is activated by androgen hormones. Once activated, AR is transported from cytoplasm to nucleus and regulates gene expression as a DNA-binding transcription factor. ERNAs transcribed from AR-regulated genes enhancers are known to respond to AR signaling and they are related to transcription reprogramming (16). However, there is still a possibility that eRNAs could be the potential byproduct of RNA polymerase.

Interestingly, lncRNAs themselves can act as an enhancer (18, 19). Huang *et al.* recently found that depletion of several lncRNAs located more than 1kb upstream to the protein coding genes resulted in corresponding decrease in neighboring genes including TAL1, Snai1 and Snai2 (Figure 2) (19). The increase in gene expression could be achieved through an RNA-mediated recruitment of transcriptional factors, displacement of transcriptional repressors, recruitment of a basal transcriptional factor or a chromatin-remodeling factor (19). However, the exact mechanism by which lncRNAs act to enhance gene expression is yet to be determined.

1.1.3 Post-transcriptional regulation—LncRNAs can also regulate gene expression by interfering post-transcription processing of mRNAs. Nuclear body paraspeckles are good examples of how lncRNAs regulate mRNA processing. The cell nucleus is a complex organelle containing many classes of nuclear bodies, such as ribosome biogenesis, transcription, and RNA splicing, that are involved in different nuclear activities. (20). Paraspeckles contain at least 3 RNA binding proteins and all of them contain RNA binding domains, which suggest that their function may be related to RNA modification (21). A ~8 Kb nuclear-retained poly(A)+ called CTN-RNA was found to localize to paraspekles and also to distribute throughout the nuclei (22). CTN-RNA is a counterpart of a protein coding gene, mCAT2. Interestingly, knockdown of CTN-RNA resulted in decrease in mCAT2 mRNA (22). CTN-RNA is stored in the paraspekles when mCAT2 is not immediately

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

required by the cell. However, when the cell is under stress, CTN-RNA is cleaved to mCAT2 mRNA resulting in increased mCAT2 protein expression (22). More recently, ncRNA MENε/β were found to be involved in paraspeckle assembly (23). Additionally, nuclear-enriched autosomal transcript 1 (NEAT1) lncRNA was also found to be co-localized with the paraspeckles and serve as a structural RNA (24). The 4 Kb lncRNA contains many self-complementary sequences that could either form an intramolecular structure or make hybrid with each other to create a large scaffold upon which the large nuclear body could build (24). These results suggest that many different lncRNAs can work together to regulate RNA processing (Figure 3).

LncRNAs also regulate mRNA translation by controlling mRNA stability. Nuclear-enriched lncRNA antisense to mouse ubiquitin carboxy-terminal hydrolase L1 (Uchl1) increases UCHL1 protein synthesis at a post-transcriptional level by combining with an embedded SINEB2 (short interspersed nuclear elements) and 5′ antisense region which specify the target sense mRNA (25). A SINE (short interspersed nuclear element) is also called non-LTR (long terminal repeat) or poly-A retrotransposons which changes its position within the genome. The embedded SINEB2 in UCHL1 confers the protein synthesis activation domain by base pairing. Antisense Uchl1 is a presenter of sense-antisense (S-AS) pair lncRNAs. LncRNAs also suppress mRNA translation via different mechanisms such as the case of lncRNA-p21 (26, 27). This transcript binds to CTNNB1 and JUNB mRNA and represses the translation of mRNA by displacing the polysome or inducing ribosome drop-off that results from the base-pair interaction between lncRNA and target mRNAs (26).

Several recent studies have highlighted a lncRNA-mRNA interaction which is very similar to the miRNA regulation of mRNA (28, 29). LncRNA that possess Alu elements is able to mediate mRNA decay by binding to the 3′UTR (29). Alu elements, which constitute 10% of all the human DNA sequence, are the most frequently observed repeats in human genome. Similar to the SINE, Alu element is also able to transpose in the genome. Base pairing of Alu containing lncRNA with mRNA 3′ UTR creates a STAU1, RNA degradation protein, binding site and leads to mRNA decay (30). Similar to miRNAs, one lncRNA can bind to a subset of mRNA and one mRNA can be targeted by multiple lncRNAs. LncRNAs are also reported to bind to miRNA and work as a miRNA sponge. LncRNA-ATB suppresses miR200 family and LncRNA MD1 suppresses miR-133 and miR-135 in this manner (31, 32). Therefore, this RNA-RNA interaction is considered to be another level of post transcription regulation of gene expression by lncRNAs.

As described above, lncRNAs can regulate gene expression at multiple stages by a variety of mechanisms, and it is becoming clear that aberrant expression of such lncRNAs can significantly contribute to tumor initiation and progression. In the following sections, we summarize the roles of lncRNAs by particularly focusing on breast cancer (Table 1).

2. Oncogenic lncRNAs in breast cancer

2.1 Proliferation and Apoptosis

2.1.1 H19—H19 is one of the well-characterized imprinting lncRNAs. It is a 2.3Kb transcript encoded by the maternal allele and is capable of silencing the IGF2 gene in the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

same allele (33). H19 is known to be involved in controlling embryonic growth and gene imprinting regulation (34, 35). The network of imprinted genes including IGF2 is under the control of H19 during embryogenesis (36). MBD1, a gene that maintains repressive marker (H3K9me3 modification of histone), is recruited and tethered by H19 to the target genes and that suppresses the gene expression (37). H19 is also known to be abnormally overexpressed in cells of higher tumorigenic capacity (38, 39) and it has been considered as an oncogenic RNA in breast epithelial cells (40, 41). In breast cancer cells, H19 was up-regulated in S-phase and promoter was found to be activated by E2F1 (42). H19 is also reported to be regulated by c-myc which is a widely dysregulated transcription factor in cancer of epithelial origin including breast cancer (43). C-myc binds to the conserved E-box on the promoter of H19 and facilitates transcriptional initiation of H19, whereas c-Myc down-regulates the IGF expression by binding to the E-box on the first intron (43). However, the mechanism by which C-myc distinguishes these two alleles is not known. Previous work showed that introduction of H19 into several tumor cell lines caused suppression of anchorage–independent growth, and therefore, H19 was considered as a tumor suppressor (44, 45). However, in another study, knockdown of H19 resulted in decrease in clonogenicity of cancer cells and suppression of anchorage-independent growth in breast cancer cell lines, MDA-MB231, SKBR3, T47D and A459 (40). Although the exact function of H19 in tumor cells is still unclear, it appears to play a pivotal role in the tumorigenic phenotype in breast cancer. The mechanism of H19 action is thought to be translational regulation; however, this needs further verification.

Interestingly, H19 was reported to antagonize let-7 microRNA which negatively regulates self-renewal of cancer stem-like cells and tumorigenicity in breast cancer (46, 47). In this study, H19 acted as a molecular sponge of Let-7 and affected the expression of endogenous let-7 targets. H19 is also known to be the precursor of miR675 which is an oncogenic miRNA and suppresses pRB expression (48, 49).

2.1.2 SRA—Steroid receptor RNA activator (SRA) is the first lncRNA discovered to function independently of epigenetic or catalytic mechanism (50). However, SRA also codes a protein (51). SRA selectively responds to hormone receptors and mediates transactivation of steroid receptor-dependent genes (52). Upon steroid stimulation, the nuclear receptor directs the assembly and stabilization of a transcription complex at the promoter of a target gene. SRA also interacts with other proteins such as SRC-1 to form a large complex which selectively enhances transcription of steroid response genes (53).

SRA has been reported to be upregulated in breast cancers (54), suggesting a potential oncogenic role. The transgenic MMTV-SRA mouse bears a human non-coding SRA sequence under the control of the mouse mammary tumor virus (MMTV) promoter (50, 55). In this mice model, SRA is expressed only in female mammary gland cells, and the mice showed aberrant mammary gland formation where multilayers of epithelial cells, ductal luminal hyperplasia and lymphocyte infiltration were observed (55). Overexpression of SRA also promoted cell proliferation and differentiation (56). Although these results suggest the potential oncogenic role of SRA, the transgenic mice exhibited normal life span, indicating that the overexpression of SRA is not sufficient to induce full blown breast cancer.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

2.1.3 LSINCT5—LSINCT5 is a 2.6KB stress-induced antisense lncRNA. Normally, LSINCT5 localizes to the nucleus and it is potentially transcribed by RNA polymerase III instead of RNA polymerase II (57, 58). LSINCT5 is overexpressed in various breast cancer cell lines and tumor tissues compared to normal breast epithelial cells (57). Knockdown of LSINCT5 resulted in decrease in cell proliferation in breast tumor cell lines. Smith *et al* found 95 potential targets of LSINCT5 by Affymetrix array, and that two genes, lncRNA NEAT1 and protein coding gene PSPC1, were most significantly regulated by LSINCT5 (57). However, these targets were not experimentally validated and the mechanism of function is yet to be known. Nevertheless, LSINCT5 is considered to be a potential oncogenic lncRNA due to its effect on cellular proliferation which warrants further study of this otherwise interesting lncRNA.

2.1.4 Zfas1—Zfas1 is the antisense to the 5′end of the protein-coding gene, Znfx1, and host snoRNAs (59). Previously, Zfas1 was only considered as a vehicle to generate snoRNAs. However, it was later found to localize within the ducts and alveoli of mammary gland and appeared to be involved in mammary gland development as it was found to be differentially expressed between pregnancy and lactation. Knockdown of Zfas1 in breast epithelial cells resulted in increased cell proliferation, and Zfas1 expression in breast cancer cells is reduced relative to normal breast tissues (59). Therefore, Zfas1 is a novel and potential breast cancer suppressor which requires future research to clarify its specific function and mechanism in breast tumorigenesis.

2.1.5 LncRNA-Smad7—LncRNA-Smad is recently identified as located adjacent to the mouse Smad7 gene (60). The expression of LncRNA-Smad7 is induced by TGF-beta in all mammary gland epithelial cells and breast cancer cell lines (60). Suppression of this lncRNA neutralized the anti-apoptosis function of TGF-β. In contrast, ectopic expression of LncRNA-Smad7 rescued apoptosis induced by a TGF-β receptor inhibitor (60). But the contribution of this lncRNA appears to be restricted to apoptosis since knockdown of it didn't affect TGF-β-induced epithelial to mesenchymal transition, phosphorylation of Smad2 or expression of the Smad7 gene (60). This finding suggests a tumorigenic role of this lncRNA, although more detailed mechanism needs to be further clarified.

2.1.6 LOC554202—LncRNA, LOC554202, is known as the host of miR31(61). Shi *et al.* found that LOC554202 expression was significantly overexpressed in breast cancer tissues and cell lines compared to normal breast tissues(62). Knockdown of LOC554202 decreased breast cancer cell proliferation, induced apoptosis and inhibited migration/invasion *in vitro* and tumorigenesis *in vivo*. However, another study showed that the LOC554202 expression was suppressed in triple negative breast cancer and that promoter hyper-methylation was the major mechanism of silencing this lncRNA (61). Therefore, the role of LOC554202 in breast tumor remains controversial.

2.1.7 UCA1—Urothelial carcinoma-associated 1 (UCA1) is a 1.4Kb lncRNA originally identified in bladder cancer. Huang *et al.* found that UCA1 promotes breast cancer cell growth both *in vitro* and *in vivo* by suppressing p27, a well-known tumor suppressorr which inhibit cyclin-dependent kinase. Binding of hnRNPI which is a well-known RNA binding

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

protein enhances p27 translation. However, UCA1 also binds to hnRNPI and competes with p27. Thus, p27 protein level is suppressed by UCA1 by requesting hnRNPI. UCA1 has an oncogenic role in breast cancer progression (63).

2.2 Invasion and metastasis

2.2.1 HOTAIR—HOX transcript antisense RNA (HOTAIR) is a 2.2Kb transcript derived from the HOXC gene cluster (10). HOTAIR is considered to be an epigenetic regulator and its function and mechanism are relatively well studied. It is the first lncRNA found to work in trans-acting manner (10). The 5′ end of HOTAIR is known to interact with the PRC2 complex, and the 3′ end of this RNA is capable of binding to the histone demethylase LSD1 (63, 64). HOTAIR guides and also serve as a scaffold for PRC2 and LSD1 at target genes (65). An example is that HOTAIR represses transcription in trans across 40Kb of the HOXD locus by recruiting the PRC2 complex which maintains the histone H3 lysine-27 trimethylation of the locus (10, 65, 66).

The HOX genes contain both tumor suppressive genes and oncogenic genes in breast cancer (67). There are numerous lncRNAs transcribed from this locus; however, their biochemical functions are not well characterized. HOTAIR is one of the oncogenic lncRNAs and may be an only example of a global regulatory phenomenon. Gupta *et al.* found that HOTAIR expression is upregulated from hundreds to nearly two thousands fold in metastatic breast cancer tissues, whereas HOTAIR expression is often high but heterogeneous in primary breast cancer (68). The expression of HOTAIR in primary breast cancer is a powerful predictor of metastasis and survival (68, 69). Furthermore, ectopic expression of HOTAIR was shown to enhance colony formation in soft agar and increase invasion through matrigel (68). The ectopic expression also modestly increased tumor growth and promoted spontaneous lung metastasis as well as lung colonization *in vivo* (68). Depletion of HOTAIR in breast cancer cells significantly decreased matrix invasiveness (68). Mechanistically, ectopic expression of HOTAIR is known to induce localization of PRC2 and H3K27me3 on 854 genes that are mostly down-regulated by HOTAIR (68). The PRC2 expression pattern in HOTAIR-overexpressed cells resembles the pattern of embryonic fibroblast expression, suggesting that HOTAIR reprograms the PRC binding pattern in breast cancer to that of embryonic fibroblast (68). These findings indicate that HOTAIR has an important role in cancer epigenome and could be a potential biomarker and a therapeutic target for breast cancer.

Chisholm *et al.* reported that HOTAIR expression is highly correlated with EZH2, which is the subunit of PRC2, in breast tumor tissues (70). The depletion of EZH2 and PRC2 was shown to be significantly increased in metastatic lesions compared to the paired primary breast tumor tissues (70). In addition, the correlation of expression of these two genes was found to be related to worse outcome in patients (70). Another study of clinical breast tumor samples showed that the level of genomic DNA methylation is positively correlated to HOTAIR (71), suggesting that the HOTAIR expression may be regulated by intergenic DNA methylation in breast cancer.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

2.3. Cancer stemness

2.3.1 SOX2OT—The SOX2 gene coding sequence lies within the intron of SOX2 overlapping transcript (SOX2OT) which is a long multi-exon lncRNA (72). Expression of SOX2 and SOX2OT is positively correlated in breast cancer, and SOX2OT is differentially expressed between ER positive and ER negative breast cancer (73). Both SOX2 and SOX2OT are upregulated in cancer stem like cells, and SOX2 is one of key genes in maintaining pluriopotency (74). Furthermore, ectopic expression of SOX2OT leads to dramatic increase in SOX2 expression and subsequent increase in breast cancer anchorage-independent growth (73). These results indicate the potential oncogenic role of SOX2OT in breast cancer by inducing or maintaining the expression of SOX2.

2.3.2 FAL1—LncRNA focally amplified on chromosome 1 (FAL1) was identified by Zhang's group through the global genomic analysis, and it is a potential oncogenic lncRNA in breast cancer (75). Knockdown of FAL1 significantly reduced the clonogenicity and inhibited cell proliferation and anchorage-independent growth of breast cancer cells (75). The knockdown of FAL1 also suppressed growth of subcutaneous tumor formed by MDA-MB-231 cell lines (75). Interestingly, FAL1 expression is associated with BMI1 which is a well-known stemness associated factor of cancer cells (76). Later, FAL1 was found to interact directly with BMI1 by RNA pull down assay (75). This study also revealed an 116nt region which is the major binding domain for BMI1 (75). Moreover, the knockdown of FAL1 significantly decreased the protein level but not the mRNA level of BMI1, suggesting that FAL1 regulates BMI1 at a post translational level by stabilizing the BMI1 protein (75). FAL1 was also found to regulate the transcription of a large set of genes that are important for cellular proliferation, death and survival as well as cellular movement and protein degradation. One of these genes is p21 which functions as a key tumor suppressor in many cancer types (75).

3. Tumor suppressive lncRNAs in breast cancer

3.1Proliferation and Apoptosis

3.1.1 GAS5—The growth arrest-specific 5(GAS5) is a non-coding RNA which plays a critical role in controlling mammalian cell apoptosis as well as proliferation (77). GAS5 was originally identified in mouse NIH 3T3 cells and it was specifically expressed when cells were under growth arrest (78). The GAS5 gene hosts several small nucleoli RNAs and is subject to complex post-transcriptional processing (77).

The expression of GAS5 was found to be significantly down-regulated in breast cancer cells compared to unaffected normal adjacent cells (79). Williams's *et al.* have recently reported that overexpression of GAS5 in MCF10A and MCF7 cells enhanced apoptosis that was mediated by UV irradiation and cisplatin (79). Consistent with their observation, Ozgur *et al.* also reported that GAS5 was up regulated in MCF7 cells during genotoxic stress-induced apoptosis (80). In some cases, ectopic expression of GAS5 alone causes growth arrest and induces apoptosis in breast cell lines. Furthermore, cells under growth arrest due to a lack of nutrients or growth factors were found to express a high level of GAS5 which sensitize cells to apoptosis (81). Kino *et al.* found that GAS5 bound to the DNA binding domain of the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

glucocorticoid receptor (GR) and competing with glucocorticoid response element (GRE) which is a regulatory sequence in the genome (77). Thus, GREs are sequestered and glucocorticoid-mediated induction of several responsive genes are suppressed including cellular inhibitor of apoptosis 2 (cIAP2) (77). GAS5 was also found to be regulated by miR21 which is a well-studied oncogenic micro RNA (82). MiR21 regulates numerous genes involved in cell growth and apoptosis, and the tumor suppressor, PTEN, is its well validated target (83). Using a qRT-PCR based array, Zhang *et al.* found that GAS5 was significantly reduced in MCF7 cells with miR21 knockdown compared to the control MCF7 cells (82). In fact, a complementary region with miR21 was identified in the GAS5 sequence (82). GAS5 expression was also found to be negatively correlated with miR21 expression in clinical specimens of breast cancer (82). Interestingly, when GAS5 was knocked-down, the miR21 expression elevated and ectopic expression of GAS5 decreased miR21 expression (82), suggesting reciprocal suppression of miR21 by GAS5. This suppression was regulated by RISC which implies that factors involved in micro RNA biogenesis or processing also regulates microRNA and lncRNA expression (82, 84). These studies indicated that GAS5 is a potential tumor suppressor which impacts the stress-induced cell growth inhibition, apoptosis and tumor invasion.

3.2 Invasion and metastasis

3.2.1 XIST—XIST is critical for the X chromosome inactivation in embryogenic development. The 17Kb long lncRNA spreads along the X chromosome and recruits PRC to maintain silencing of the X chromosome (85). A recent study by Jeon et.al. showed that YY1 guides XIST loading and spreading during the initial phases of X chromosome inactivation (86). This dosage compensation resulted in silencing of more than 1000 genes on one of the X chromosome (86).

The role of XIST in breast cancer has been intensively studied but remains controversial and unclear. The Livingston group showed that BRCA1, a breast tumor suppressor, supported XIST RNA concentration on the inactive X chromosome (87). BRCA1 was found to co-localize with inactive X chromosome, and plays a critical role in maintaining markers of X chromosome inactivation (87). This observation suggests that loss of BRCA1 in female cells may lead to destabilization of X chromosome. However, Xiao *et al.* found that XIST functions independently of BRCA1 in X inactivation (88). According to results of Livingston group, the loss of BRCA1 in female cells could reactivate genes on the inactive X chromosome due to loss of XIST (87); however, the reason of this apparent controversy is not clear. They also found that BRCA1 was not enriched on XIST RNA-coated chromatin of inactive X chromosome (88). To respond to these findings, Livingston group showed further evidences of BRCA1’s communication with inactive X chromosome (89). The interaction between BRCA1 and XIST remains an issue of debate (90, 91); however, it is of great importance to understand the underlying mechanism by which XIST interact with BRCA1.

In another study, the expression of XIST was found to differ between subtypes of breast cancer and also related to BRCA1 status (92). Only the XIST, which was expressed from the inactive X chromosome, was regulated by BRCA1 (92). However, XIST can also be abnormally expressed from an active X chromosome (93). Although most breast tumor cell

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

lines lost the inactive X chromosome and gained one more active X chromosome, whether loss of XIST is leading to the loss of inactivated X chromosome or loss of XIST leads to the loss of inactive X chromosome is not clear (93). These results indicate that the mechanism of XIST expression in breast cancer cells may be different from that of the normal cells.

Andrea *et al.* recently reported that XIST misbehaved particularly in basal like breast cancer (BLC) cells (94). In this study, 80% of the samples showed X chromosome abnormality, and duplication of the active X chromosome and loss of X inactivation center were most frequently seen in these samples (94). However, there were only 2 out 20 samples that lost their inactive X chromosome in non-BLC patients (94). This misbehavior of XIST is not related to BRCA1 because most samples retained wild type BRCA. Surprisingly, the expressions of X linked genes were not globally upregulated in those BLC samples (94). There were only few loci that escaped X chromosome inactivation in the samples that lost XIST. These studies suggest that X chromosome abnormalities may contribute to the BLC formation; although the mechanism and effect of these abnormalities are yet to be understood. A future study is needed to clarify the relation of XIST and X chromosome abnormalities to understand the pathological role of this lncRNA in breast cancer.

4. Future direction

Due to the rapid development of high-throughput sequencing technique and genomic profiling along with the powerful bioinformatics tools, significant amount of information about lncRNAs have been emerged in the last decade (7). Comprehensive sequencing analysis has been a useful tool to identify novel lncRNAs and to discover new roles of lncRNAs by providing a framework for the current lncRNA studies. Maruyama *et al.* recently analyzed transcriptome from normal and breast cancer epithelial cells by specifically focusing on the distribution of sense and antisense lncRNA between normal and cancer (95). Many of the differentially expressed antisense transcripts likely represent lncRNAs. Certain genes only generate antisense transcripts in normal or cancer and they are involved in important physiological function such as tumor cell metabolism. Zhao *et al.* comprehensively characterized the lncRNAs between different breast cancer subtypes (96). They observed 20 lncRNA genes that are significantly correlated with breast cancer subtypes and 14 lncRNAs significantly correlated with breast cancer grades. They also identified 3 lncRNAs (LINC00324, PTPRG-AS1 and SNHG17) that were significantly correlated with clinical outcomes of patients (96). Su *et al.* also analyzed lncRNAs in breast cancer subtypes and classified lncRNAs into 4 subtypes with different prognoses (97). Furthermore, Reiche *et al.* analyzed microarray data of mRNA from clinical specimen and identified 19,000 transcripts that were significantly and differentially expressed between normal and cancer breast tissue (98). Interestingly, they found that more than half of these transcripts were lncRNAs. A number of these genes have also been identified in relation to cancer associated protein coding genes. Although their biological function in breast cancer is not yet clear, both computational and experimental analysis should address the molecular mechanism of lncRNAs.

Breast cancer is known to be driven by many different oncogenic pathways, and there are multiple steps that are involved in cancer progression and invasion (99). Therefore,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

screening based on functional assays could also identify novel lncRNAs that are involved in tumor progression. In addition, it is well established that tumor microenvironmental cells play pivotal roles in regulating breast cancer progression (100). However, the roles of lncRNA in these cells are not well explored. It should be noted that lncRNAs are also secreted in exosome by cancer cells, which indicates that lncRNA may play a critical role in the communication of cancer cells and microenvironmental cells and metastasis niche formation (101, 102).

LncRNAs represent a novel and rarely characterized components of the cancer cells, and therefore, there is a great potential to develop biomarkers using these RNAs for clinical use (103, 104). An ideal biomarker should be easily accessible and is particularly desirable if it can be sampled from body fluids, such as serum or urine. LncRNAs are indeed detected in human body fluids. At present, no lncRNAs have been characterized as potential biomarkers for breast cancer in human body fluids. But there are few lncRNAs characterized as biomarkers in other cancers such as hepatocarcinoma and prostate cancer (105, 106). However, there are few shortcomings to use lncRNAs as biomarkers. The most concerned question is the stability of lncRNA in human bodily fluids since RNAs are considered to be unstable. But lncRNAs are often packed into micro-vesicular particles such as exosomes which may protect them from degradation. As lncRNAs play important role in breast cancer progression, they are potential novel therapeutic targets. Future development of lncRNA-based therapy is not on sight right now and RNAs are not considered to be good target with current technology; however, the recent breakthrough of CRISPR system for genome editing *in vivo* (107–109) holds great promise for targeting lncRNAs for cancer treatment.

References

1. Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, Stadler PF, Hertel J, Hackermuller J, Hofacker IL, Bell I, Cheung E, Drenkow J, Dumais E, Patel S, Helt G, Ganesh M, Ghosh S, Piccolboni A, Sementchenko V, Tammana H, Gingeras TR. RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science. 2007; 316(5830):1484–8. doi: 1138341 [pii]. [PubMed: 17510325]
2. Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, Oyama R, Ravasi T, Lenhard B, Wells C, Kodzius R, Shimokawa K, Bajic VB, Brenner SE, Batalov S, et al. The transcriptional landscape of the mammalian genome. Science. 2005; 309(5740):1559–63. doi:309/5740/1559 [pii]. [PubMed: 16141072]
3. Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, Cabili MN, Jaenisch R, Mikkelsen TS, Jacks T, Hacohen N, Bernstein BE, Kellis M, Regev A, Rinn JL, Lander ES. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature. 2009; 458(7235):223–7. DOI: 10.1038/nature07672 [PubMed: 19182780]
4. Gerstein MB, Kundaje A, Hariharan M, Landt SG, Yan KK, Cheng C, Mu XJ, Khurana E, Rozowsky J, Alexander R, Min R, Alves P, Abyzov A, Addleman N, Bhardwaj N, Boyle AP, Cayting P, Charos A, Chen DZ, Cheng Y, Clarke D, Eastman C, Euskirchen G, Frietze S, Fu Y, Gertz J, Grubert F, Harmanci A, Jain P, Kasowski M, Lacroute P, Leng J, Lian J, Monahan H, O'Geen H, Ouyang Z, Partridge EC, Patacsil D, Pauli F, Raha D, Ramirez L, Reddy TE, Reed B, Shi M, Slifer T, Wang J, Wu L, Yang X, Yip KY, Zilberman-Schapira G, Batzoglou S, Sidow A, Farnham PJ, Myers RM, Weissman SM, Snyder M. Architecture of the human regulatory network derived from ENCODE data. Nature. 2012; 489(7414):91–100. DOI: 10.1038/nature11245 [PubMed: 22955619]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

5. Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, Rinn JL. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev. 2011; 25(18):1915–27. DOI: 10.1101/gad.17446611 [PubMed: 21890647]

6. Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, Thomas K, Presser A, Bernstein BE, van Oudenaarden A, Regev A, Lander ES, Rinn JL. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci U S A. 2009; 106(28):11667–72. DOI: 10.1073/pnas.0904715106 [PubMed: 19571010]

7. Amaral PP, Clark MB, Gascoigne DK, Dinger ME, Mattick JS. lncRNAdb: a reference database for long noncoding RNAs. Nucleic Acids Res. 2011; 39:D146–51. (Database issue). DOI: 10.1093/nar/gkq1138 [PubMed: 21112873]

8. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. Cell. 2007; 128(4):669–81. doi:S0092-86740700128-6 [pii]. [PubMed: 17320505]

9. Baylin SB, Ohm JE. Epigenetic gene silencing in cancer - a mechanism for early oncogenic pathway addiction? Nat Rev Cancer. 2006; 6(2):107–16. doi:nrc1799 [pii]. [PubMed: 16491070]

10. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E, Chang HY. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell. 2007; 129(7):1311–23. doi:S0092-86740700659-9 [pii]. [PubMed: 17604720]

11. Lee JT. Epigenetic regulation by long noncoding RNAs. Science. 2012; 338(6113):1435–9. DOI: 10.1126/science.1231776 [PubMed: 23239728]

12. Tan J, Yang X, Zhuang L, Jiang X, Chen W, Lee PL, Karuturi RK, Tan PB, Liu ET, Yu Q. Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. Genes Dev. 2007; 21(9):1050–63. doi:gad.1524107 [pii]. [PubMed: 17437993]

13. Mattick JS. The genetic signatures of noncoding RNAs. PLoS Genet. 2009; 5(4):e1000459.doi: 10.1371/journal.pgen.1000459 [PubMed: 19390609]

14. Chu C, Qu K, Zhong FL, Artandi SE, Chang HY. Genomic maps of long noncoding RNA occupancy reveal principles of RNA-chromatin interactions. Mol Cell. 2011; 44(4):667–78. DOI: 10.1016/j.molcel.2011.08.027 [PubMed: 21963238]

15. Kim TK, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, Harmin DA, Laptewicz M, Barbara-Haley K, Kuersten S, Markenscoff-Papadimitriou E, Kuhl D, Bito H, Worley PF, Kreiman G, Greenberg ME. Widespread transcription at neuronal activity-regulated enhancers. Nature. 2010; 465(7295):182–7. DOI: 10.1038/nature09033 [PubMed: 20393465]

16. Wang D, Garcia-Bassets I, Benner C, Li W, Su X, Zhou Y, Qiu J, Liu W, Kaikkonen MU, Ohgi KA, Glass CK, Rosenfeld MG, Fu XD. Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. Nature. 2011; 474(7351):390–4. DOI: 10.1038/nature10006 [PubMed: 21572438]

17. Melo CA, Drost J, Wijchers PJ, van de Werken H, de Wit E, Oude Vrielink JA, Elkon R, Melo SA, Leveille N, Kalluri R, de Laat W, Agami R. eRNAs are required for p53-dependent enhancer activity and gene transcription. Mol Cell. 2013; 49(3):524–35. DOI: 10.1016/j.molcel.2012.11.021 [PubMed: 23273978]

18. Yang F, Zhang L, Huo XS, Yuan JH, Xu D, Yuan SX, Zhu N, Zhou WP, Yang GS, Wang YZ, Shang JL, Gao CF, Zhang FR, Wang F, Sun SH. Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. Hepatology. 2011; 54(5):1679–89. DOI: 10.1002/hep.24563 [PubMed: 21769904]

19. Orom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, Bussotti G, Lai F, Zytnicki M, Notredame C, Huang Q, Guigo R, Shiekhattar R. Long noncoding RNAs with enhancer-like function in human cells. Cell. 2010; 143(1):46–58. DOI: 10.1016/j.cell.2010.09.001 [PubMed: 20887892]

20. Bond CS, Fox AH. Paraspeckles: nuclear bodies built on long noncoding RNA. J Cell Biol. 2009; 186(5):637–44. DOI: 10.1083/jcb.200906113 [PubMed: 19720872]

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

21. Mao YS, Sunwoo H, Zhang B, Spector DL. Direct visualization of the co-transcriptional assembly of a nuclear body by noncoding RNAs. Nat Cell Biol. 2011; 13(1):95–101. DOI: 10.1038/ncb2140 [PubMed: 21170033]
22. Prasanth KV, Prasanth SG, Xuan Z, Hearn S, Freier SM, Bennett CF, Zhang MQ, Spector DL. Regulating gene expression through RNA nuclear retention. Cell. 2005; 123(2):249–63. doi:S0092-86740500870-6 [pii]. [PubMed: 16239143]
23. Sasaki YT, Ideue T, Sano M, Mituyama T, Hirose T. MENepsilon/beta noncoding RNAs are essential for structural integrity of nuclear paraspeckles. Proc Natl Acad Sci U S A. 2009; 106(8): 2525–30. DOI: 10.1073/pnas.0807899106 [PubMed: 19188602]
24. Clemson CM, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, Chess A, Lawrence JB. An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. Mol Cell. 2009; 33(6):717–26. DOI: 10.1016/j.molcel.2009.01.026 [PubMed: 19217333]
25. Carrieri C, Cimatti L, Biagioli M, Beugnet A, Zucchelli S, Fedele S, Pesce E, Ferrer I, Collavin L, Santoro C, Forrest AR, Carninci P, Biffo S, Stupka E, Gustincich S. Long non-coding antisense RNA controls Uchl1 translation through an embedded SINEB2 repeat. Nature. 2012; 491(7424): 454–7. DOI: 10.1038/nature11508 [PubMed: 23064229]
26. Yoon JH, Abdelmohsen K, Srikantan S, Yang X, Martindale JL, De S, Huarte M, Zhan M, Becker KG, Gorospe M. LincRNA-p21 suppresses target mRNA translation. Mol Cell. 2012; 47(4):648–55. DOI: 10.1016/j.molcel.2012.06.027 [PubMed: 22841487]
27. Wu G, Cai J, Han Y, Chen J, Huang ZP, Chen C, Cai Y, Huang H, Yang Y, Liu Y, Xu Z, He D, Zhang X, Hu X, Pinello L, Zhong D, He F, Yuan GC, Wang DZ, Zeng C. LincRNA-p21 Regulates Neointima Formation, Vascular Smooth Muscle Cell Proliferation, Apoptosis, and Atherosclerosis by Enhancing p53 Activity. Circulation. 2014; 130(17):1452–65. DOI: 10.1161/CIRCULATIONAHA.114.011675 [PubMed: 25156994]
28. Wang K, Sun T, Li N, Wang Y, Wang JX, Zhou LY, Long B, Liu CY, Liu F, Li PF. MDRL lncRNA regulates the processing of miR-484 primary transcript by targeting miR-361. PLoS Genet. 2014; 10(7):e1004467.doi: 10.1371/journal.pgen.1004467 [PubMed: 25057983]
29. Wang J, Gong C, Maquat LE. Control of myogenesis by rodent SINE-containing lncRNAs. Genes Dev. 2013; 27(7):793–804. DOI: 10.1101/gad.212639.112 [PubMed: 23558772]
30. Park E, Maquat LE. Staufen-mediated mRNA decay. Wiley Interdiscip Rev RNA. 2013; 4(4):423–35. DOI: 10.1002/wrna.1168 [PubMed: 23681777]
31. Yuan JH, Yang F, Wang F, Ma JZ, Guo YJ, Tao QF, Liu F, Pan W, Wang TT, Zhou CC, Wang SB, Wang YZ, Yang Y, Yang N, Zhou WP, Yang GS, Sun SH. A long noncoding RNA activated by TGF-beta promotes the invasion-metastasis cascade in hepatocellular carcinoma. Cancer Cell. 2014; 25(5):666–81. DOI: 10.1016/j.ccr.2014.03.010 [PubMed: 24768205]
32. Legnini I, Morlando M, Mangiavacchi A, Fatica A, Bozzoni I. A feedforward regulatory loop between HuR and the long noncoding RNA linc-MD1 controls early phases of myogenesis. Mol Cell. 2014; 53(3):506–14. DOI: 10.1016/j.molcel.2013.12.012 [PubMed: 24440503]
33. Edwards CA, Ferguson-Smith AC. Mechanisms regulating imprinted genes in clusters. Curr Opin Cell Biol. 2007; 19(3):281–9. doi:S0955-06740700065-8 [pii]. [PubMed: 17467259]
34. Gabory A, Jammes H, Dandolo L. The H19 locus: role of an imprinted non-coding RNA in growth and development. Bioessays. 2010; 32(6):473–80. DOI: 10.1002/bies.200900170 [PubMed: 20486133]
35. Feil R. Epigenetic asymmetry in the zygote and mammalian development. Int J Dev Biol. 2009; 53(2–3):191–201. DOI: 10.1387/ijdb.082654rf [PubMed: 19378254]
36. Monnier P, Martinet C, Pontis J, Stancheva I, Ait-Si-Ali S, Dandolo L. H19 lncRNA controls gene expression of the Imprinted Gene Network by recruiting MBD1. Proc Natl Acad Sci U S A. 2013; 110(51):20693–8. DOI: 10.1073/pnas.1310201110 [PubMed: 24297921]
37. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nat Rev Genet. 2002; 3(6):415–28. DOI: 10.1038/nrg816 [PubMed: 12042769]
38. Cui H, Onyango P, Brandenburg S, Wu Y, Hsieh CL, Feinberg AP. Loss of imprinting in colorectal cancer linked to hypomethylation of H19 and IGF2. Cancer Res. 2002; 62(22):6442–6. [PubMed: 12438232]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

39. Hibi K, Nakamura H, Hirai A, Fujikake Y, Kasai Y, Akiyama S, Ito K, Takagi H. Loss of H19 imprinting in esophageal cancer. Cancer Res. 1996; 56(3):480–2. [PubMed: 8564957]
40. Lottin S, Adriaenssens E, Dupressoir T, Berteaux N, Montpellier C, Coll J, Dugimont T, Curgy JJ. Overexpression of an ectopic H19 gene enhances the tumorigenic properties of breast cancer cells. Carcinogenesis. 2002; 23(11):1885–95. [PubMed: 12419837]
41. van Roozendaal CE, Gillis AJ, Klijn JG, van Ooijen B, Claassen CJ, Eggermont AM, Henzen-Logmans SC, Oosterhuis JW, Foekens JA, Looijenga LH. Loss of imprinting of IGF2 and not H19 in breast cancer, adjacent normal tissue and derived fibroblast cultures. FEBS Lett. 1998; 437(1–2):107–11. doi:S0014-57939801211-3 [pii]. [PubMed: 9804181]
42. Berteaux N, Lottin S, Monte D, Pinte S, Quatannens B, Coll J, Hondermarck H, Curgy JJ, Dugimont T, Adriaenssens E. H19 mRNA-like noncoding RNA promotes breast cancer cell proliferation through positive control by E2F1. J Biol Chem. 2005; 280(33):29625–36. doi:M504033200 [pii]. [PubMed: 15985428]
43. Barsyte-Lovejoy D, Lau SK, Boutros PC, Khosravi F, Jurisica I, Andrulis IL, Tsao MS, Penn LZ. The c-Myc oncogene directly induces the H19 noncoding RNA by allele-specific binding to potentiate tumorigenesis. Cancer Res. 2006; 66(10):5330–7. doi:66/10/5330 [pii]. [PubMed: 16707459]
44. Yoshimizu T, Miroglio A, Ripoche MA, Gabory A, Vernucci M, Riccio A, Colnot S, Godard C, Terris B, Jammes H, Dandolo L. The H19 locus acts in vivo as a tumor suppressor. Proc Natl Acad Sci U S A. 2008; 105(34):12417–22. DOI: 10.1073/pnas.0801540105 [PubMed: 18719115]
45. Hao Y, Crenshaw T, Moulton T, Newcomb E, Tycko B. Tumour-suppressor activity of H19 RNA. Nature. 1993; 365(6448):764–7. DOI: 10.1038/365764a0 [PubMed: 7692308]
46. Kallen AN, Zhou XB, Xu J, Qiao C, Ma J, Yan L, Lu L, Liu C, Yi JS, Zhang H, Min W, Bennett AM, Gregory RI, Ding Y, Huang Y. The imprinted H19 lncRNA antagonizes let-7 microRNAs. Mol Cell. 2013; 52(1):101–12. DOI: 10.1016/j.molcel.2013.08.027 [PubMed: 24055342]
47. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, Huang Y, Hu X, Su F, Lieberman J, Song E. let-7 regulates self renewal and tumorigenicity of breast cancer cells. Cell. 2007; 131(6):1109–23. doi:S0092-86740701417-1 [pii]. [PubMed: 18083101]
48. Keniry A, Oxley D, Monnier P, Kyba M, Dandolo L, Smits G, Reik W. The H19 linc RNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. Nat Cell Biol. 2012; 14(7): 659–65. DOI: 10.1038/ncb2521 [PubMed: 22684254]
49. Tsang WP, Ng EK, Ng SS, Jin H, Yu J, Sung JJ, Kwok TT. Oncofetal H19-derived miR-675 regulates tumor suppressor RB in human colorectal cancer. Carcinogenesis. 2010; 31(3):350–8. DOI: 10.1093/carcin/bgp181 [PubMed: 19926638]
50. Novikova IV, Hennelly SP, Sanbonmatsu KY. Structural architecture of the human long non-coding RNA, steroid receptor RNA activator. Nucleic Acids Res. 2012; 40(11):5034–51. DOI: 10.1093/nar/gks071 [PubMed: 22362738]
51. Sharif J, Muto M, Takebayashi S, Suetake I, Iwamatsu A, Endo TA, Shinga J, Mizutani-Koseki Y, Toyoda T, Okamura K, Tajima S, Mitsuya K, Okano M, Koseki H. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. Nature. 2007; 450(7171):908–12. doi:nature06397 [pii]. [PubMed: 17994007]
52. Vicent GP, Nacht AS, Zaurin R, Font-Mateu J, Soronellas D, Le Dily F, Reyes D, Beato M. Unliganded progesterone receptor-mediated targeting of an RNA-containing repressive complex silences a subset of hormone-inducible genes. Genes Dev. 2013; 27(10):1179–97. DOI: 10.1101/gad.215293.113 [PubMed: 23699411]
53. Lanz RB, McKenna NJ, Onate SA, Albrecht U, Wong J, Tsai SY, Tsai MJ, O'Malley BW. A steroid receptor coactivator, SRA, functions as an RNA and is present in an SRC-1 complex. Cell. 1999; 97(1):17–27. doi:S0092-86740080711-4 [pii]. [PubMed: 10199399]
54. Cooper C, Guo J, Yan Y, Chooniedass-Kothari S, Hube F, Hamedani MK, Murphy LC, Myal Y, Leygue E. Increasing the relative expression of endogenous non-coding Steroid Receptor RNA Activator (SRA) in human breast cancer cells using modified oligonucleotides. Nucleic Acids Res. 2009; 37(13):4518–31. DOI: 10.1093/nar/gkp441 [PubMed: 19483093]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

55. Colley SM, Leedman PJ. Steroid Receptor RNA Activator - A nuclear receptor coregulator with multiple partners: Insights and challenges. Biochimie. 2011; 93(11):1966–72. DOI: 10.1016/j.biochi.2011.07.004 [PubMed: 21807064]
56. Lanz RB, Chua SS, Barron N, Soder BM, DeMayo F, O'Malley BW. Steroid receptor RNA activator stimulates proliferation as well as apoptosis in vivo. Mol Cell Biol. 2003; 23(20):7163–76. [PubMed: 14517287]
57. Silva JM, Boczek NJ, Berres MW, Ma X, Smith DI. LSINCT5 is over expressed in breast and ovarian cancer and affects cellular proliferation. RNA Biol. 2011; 8(3):496–505. doi:14800 [pii]. [PubMed: 21532345]
58. Dieci G, Fiorino G, Castelnuovo M, Teichmann M, Pagano A. The expanding RNA polymerase III transcriptome. Trends Genet. 2007; 23(12):614–22. doi:S0168-95250700307-1 [pii]. [PubMed: 17977614]
59. Askarian-Amiri ME, Crawford J, French JD, Smart CE, Smith MA, Clark MB, Ru K, Mercer TR, Thompson ER, Lakhani SR, Vargas AC, Campbell IG, Brown MA, Dinger ME, Mattick JS. SNORD-host RNA Zfas1 is a regulator of mammary development and a potential marker for breast cancer. RNA. 2011; 17(5):878–91. DOI: 10.1261/rna.2528811 [PubMed: 21460236]
60. Arase M, Horiguchi K, Ehata S, Morikawa M, Tsutsumi S, Aburatani H, Miyazono K, Koinuma D. Transforming growth factor-beta-induced lncRNA-Smad7 inhibits apoptosis of mouse breast cancer JygMC(A) cells. Cancer Sci. 2014; 105(8):974–82. DOI: 10.1111/cas.12454 [PubMed: 24863656]
61. Augoff K, McCue B, Plow EF, Sossey-Alaoui K. miR-31 and its host gene lncRNA LOC554202 are regulated by promoter hypermethylation in triple-negative breast cancer. Mol Cancer. 2012; 11:5.doi: 10.1186/1476-4598-11-5 [PubMed: 22289355]
62. Shi Y, Lu J, Zhou J, Tan X, He Y, Ding J, Tian Y, Wang L, Wang K. Long non-coding RNA Loc554202 regulates proliferation and migration in breast cancer cells. Biochem Biophys Res Commun. 2014; 446(2):448–53. DOI: 10.1016/j.bbrc.2014.02.144 [PubMed: 24631686]
63. Huang J, Zhou N, Watabe K, Lu Z, Wu F, Xu M, Mo YY. Long non-coding RNA UCA1 promotes breast tumor growth by suppression of p27 (Kip1). Cell death & disease. 2014; 5:1.doi: 10.1038/cddis.2013.541
64. He S, Liu S, Zhu H. The sequence, structure and evolutionary features of HOTAIR in mammals. BMC Evol Biol. 2011; 11:102.doi: 10.1186/1471-2148-11-102 [PubMed: 21496275]
65. Wu L, Murat P, Matak-Vinkovic D, Murrell A, Balasubramanian S. Binding interactions between long noncoding RNA HOTAIR and PRC2 proteins. Biochemistry. 2013; 52(52):9519–27. DOI: 10.1021/bi401085h [PubMed: 24320048]
66. Kogo R, Shimamura T, Mimori K, Kawahara K, Imoto S, Sudo T, Tanaka F, Shibata K, Suzuki A, Komune S, Miyano S, Mori M. Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. Cancer Res. 2011; 71(20):6320–6. DOI: 10.1158/0008-5472.CAN-11-1021 [PubMed: 21862635]
67. Cantile M, Pettinato G, Procino A, Feliciello I, Cindolo L, Cillo C. In vivo expression of the whole HOX gene network in human breast cancer. Eur J Cancer. 2003; 39(2):257–64. doi:S0959804902005993 [pii]. [PubMed: 12509959]
68. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature. 2010; 464(7291):1071–6. DOI: 10.1038/nature08975 [PubMed: 20393566]
69. Sorensen KP, Thomassen M, Tan Q, Bak M, Cold S, Burton M, Larsen MJ, Kruse TA. Long non-coding RNA HOTAIR is an independent prognostic marker of metastasis in estrogen receptor-positive primary breast cancer. Breast Cancer Res Treat. 2013; 142(3):529–36. DOI: 10.1007/s10549-013-2776-7 [PubMed: 24258260]
70. Chisholm KM, Wan Y, Li R, Montgomery KD, Chang HY, West RB. Detection of long non-coding RNA in archival tissue: correlation with polycomb protein expression in primary and metastatic breast carcinoma. PLoS ONE. 2012; 7(10):e47998.doi: 10.1371/journal.pone.0047998 [PubMed: 23133536]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

71. Lu L, Zhu G, Zhang C, Deng Q, Katsaros D, Mayne ST, Risch HA, Mu L, Canuto EM, Gregori G, Benedetto C, Yu H. Association of large noncoding RNA HOTAIR expression and its downstream intergenic CpG island methylation with survival in breast cancer. Breast Cancer Res Treat. 2012; 136(3):875–83. DOI: 10.1007/s10549-012-2314-z [PubMed: 23124417]
72. Amaral PP, Neyt C, Wilkins SJ, Askarian-Amiri ME, Sunkin SM, Perkins AC, Mattick JS. Complex architecture and regulated expression of the Sox2ot locus during vertebrate development. RNA. 2009; 15(11):2013–27. DOI: 10.1261/rna.1705309 [PubMed: 19767420]
73. Askarian-Amiri ME, Seyfoddin V, Smart CE, Wang J, Kim JE, Hansji H, Baguley BC, Finlay GJ, Leung EY. Emerging role of long non-coding RNA SOX2OT in SOX2 regulation in breast cancer. PLoS ONE. 2014; 9(7):e102140.doi: 10.1371/journal.pone.0102140 [PubMed: 25006803]
74. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R II, Slukvin, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007; 318(5858):1917–20. doi:1151526 [pii]. [PubMed: 18029452]
75. Hu X, Feng Y, Zhang D, Zhao SD, Hu Z, Greshock J, Zhang Y, Yang L, Zhong X, Wang LP, Jean S, Li C, Huang Q, Katsaros D, Montone KT, Tanyi JL, Lu Y, Boyd J, Nathanson KL, Li H, Mills GB, Zhang L. A functional genomic approach identifies FAL1 as an oncogenic long noncoding RNA that associates with BMI1 and represses p21 expression in cancer. Cancer Cell. 2014; 26(3): 344–57. DOI: 10.1016/j.ccr.2014.07.009 [PubMed: 25203321]
76. Godlewski J, Nowicki MO, Bronisz A, Williams S, Otsuki A, Nuovo G, Raychaudhury A, Newton HB, Chiocca EA, Lawler S. Targeting of the Bmi-1 oncogene/stem cell renewal factor by microRNA-128 inhibits glioma proliferation and self-renewal. Cancer Res. 2008; 68(22):9125–30. DOI: 10.1158/0008-5472.CAN-08-2629 [PubMed: 19010882]
77. Kino T, Hurt DE, Ichijo T, Nader N, Chrousos GP. Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. Sci Signal. 2010; 3(107):ra8.doi: 10.1126/scisignal.2000568 [PubMed: 20124551]
78. Schneider C, King RM, Philipson L. Genes specifically expressed at growth arrest of mammalian cells. Cell. 1988; 54(6):787–93. doi:S0092-86748891065-3 [pii]. [PubMed: 3409319]
79. Mourtada-Maarabouni M, Pickard MR, Hedge VL, Farzaneh F, Williams GT. GAS5, a non-protein-coding RNA, controls apoptosis and is downregulated in breast cancer. Oncogene. 2009; 28(2):195–208. DOI: 10.1038/onc.2008.373 [PubMed: 18836484]
80. Ozgur E, Mert U, Isin M, Okutan M, Dalay N, Gezer U. Differential expression of long non-coding RNAs during genotoxic stress-induced apoptosis in HeLa and MCF-7 cells. Clin Exp Med. 2013; 13(2):119–26. DOI: 10.1007/s10238-012-0181-x [PubMed: 22487937]
81. Piao HL, Ma L. Non-coding RNAs as regulators of mammary development and breast cancer. J Mammary Gland Biol Neoplasia. 2012; 17(1):33–42. DOI: 10.1007/s10911-012-9245-5 [PubMed: 22350981]
82. Zhang Z, Zhu Z, Watabe K, Zhang X, Bai C, Xu M, Wu F, Mo YY. Negative regulation of lncRNA GAS5 by miR-21. Cell Death Differ. 2013; 20(11):1558–68. DOI: 10.1038/cdd.2013.110 [PubMed: 23933812]
83. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology. 2007; 133(2):647–58. doi:S0016-50850701002-5 [pii]. [PubMed: 17681183]
84. Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell. 2005; 123(4):631–40. doi:S0092-86740501109-8 [pii]. [PubMed: 16271387]
85. Penny GD, Kay GF, Sheardown SA, Rastan S, Brockdorff N. Requirement for Xist in X chromosome inactivation. Nature. 1996; 379(6561):131–7. DOI: 10.1038/379131a0 [PubMed: 8538762]
86. Jeon Y, Lee JT. YY1 tethers Xist RNA to the inactive X nucleation center. Cell. 2011; 146(1):119–33. DOI: 10.1016/j.cell.2011.06.026 [PubMed: 21729784]
87. Ganesan S, Silver DP, Greenberg RA, Avni D, Drapkin R, Miron A, Mok SC, Randrianarison V, Brodie S, Salstrom J, Rasmussen TP, Klimke A, Marrese C, Marahrens Y, Deng CX, Feunteun J, Livingston DM. BRCA1 supports XIST RNA concentration on the inactive X chromosome. Cell. 2002; 111(3):393–405. doi:S0092867402010528 [pii]. [PubMed: 12419249]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

88. Xiao C, Sharp JA, Kawahara M, Davalos AR, Difilippantonio MJ, Hu Y, Li W, Cao L, Buetow K, Ried T, Chadwick BP, Deng CX, Panning B. The XIST noncoding RNA functions independently of BRCA1 in X inactivation. Cell. 2007; 128(5):977–89. doi:S0092-86740700179-1 [pii]. [PubMed: 17350580]
89. Silver DP, Dimitrov SD, Feunteun J, Gelman R, Drapkin R, Lu SD, Shestakova E, Velmurugan S, Denunzio N, Dragomir S, Mar J, Liu X, Rottenberg S, Jonkers J, Ganesan S, Livingston DM. Further evidence for BRCA1 communication with the inactive X chromosome. Cell. 2007; 128(5): 991–1002. doi:S0092-86740700249-8 [pii]. [PubMed: 17350581]
90. Pageau GJ, Hall LL, Lawrence JB. BRCA1 does not paint the inactive X to localize XIST RNA but may contribute to broad changes in cancer that impact XIST and Xi heterochromatin. J Cell Biochem. 2007; 100(4):835–50. DOI: 10.1002/jcb.21188 [PubMed: 17146760]
91. Ganesan S, Silver DP, Drapkin R, Greenberg R, Feunteun J, Livingston DM. Association of BRCA1 with the inactive X chromosome and XIST RNA. Philos Trans R Soc Lond B Biol Sci. 2004; 359(1441):123–8. DOI: 10.1098/rstb.2003.1371 [PubMed: 15065664]
92. Sirchia SM, Ramoscelli L, Grati FR, Barbera F, Coradini D, Rossella F, Porta G, Lesma E, Ruggeri A, Radice P, Simoni G, Miozzo M. Loss of the inactive X chromosome and replication of the active X in BRCA1-defective and wild-type breast cancer cells. Cancer Res. 2005; 65(6):2139–46. doi:65/6/2139 [pii]. [PubMed: 15781624]
93. Sirchia SM, Tabano S, Monti L, Recalcati MP, Gariboldi M, Grati FR, Porta G, Finelli P, Radice P, Miozzo M. Misbehaviour of XIST RNA in breast cancer cells. PLoS ONE. 2009; 4(5):e5559.doi: 10.1371/journal.pone.0005559 [PubMed: 19440381]
94. Richardson AL, Wang ZC, De Nicolo A, Lu X, Brown M, Miron A, Liao X, Iglehart JD, Livingston DM, Ganesan S. X chromosomal abnormalities in basal-like human breast cancer. Cancer Cell. 2006; 9(2):121–32. doi:S1535-61080600029-8 [pii]. [PubMed: 16473279]
95. Maruyama R, Shipitsin M, Choudhury S, Wu Z, Protopopov A, Yao J, Lo PK, Bessarabova M, Ishkin A, Nikolsky Y, Liu XS, Sukumar S, Polyak K. Altered antisense-to-sense transcript ratios in breast cancer. Proc Natl Acad Sci U S A. 2012; 109(8):2820–4. DOI: 10.1073/pnas.1010559107 [PubMed: 21098291]
96. Zhao W, Luo J, Jiao S. Comprehensive characterization of cancer subtype associated long non-coding RNAs and their clinical implications. Sci Rep. 2014; 4:6591.doi: 10.1038/srep06591 [PubMed: 25307233]
97. Su X, Malouf GG, Chen Y, Zhang J, Yao H, Valero V, Weinstein JN, Spano JP, Meric-Bernstam F, Khayat D, Esteva FJ. Comprehensive analysis of long non-coding RNAs in human breast cancer clinical subtypes. Oncotarget. 2014; 5(20):9864–9876. doi:2454 [pii]. [PubMed: 25296969]
98. Reiche K, Kasack K, Schreiber S, Luders T, Due EU, Naume B, Riis M, Kristensen VN, Horn F, Borresen-Dale AL, Hackermuller J, Baumbusch LO. Long Non-Coding RNAs Differentially Expressed between Normal versus Primary Breast Tumor Tissues Disclose Converse Changes to Breast Cancer-Related Protein-Coding Genes. PLoS ONE. 2014; 9(9):e106076.doi: 10.1371/journal.pone.0106076 [PubMed: 25264628]
99. Simpson PT, Reis-Filho JS, Gale T, Lakhani SR. Molecular evolution of breast cancer. J Pathol. 2005; 205(2):248–54. DOI: 10.1002/path.1691 [PubMed: 15641021]
100. Gout S, Huot J. Role of cancer microenvironment in metastasis: focus on colon cancer. Cancer Microenviron. 2008; 1(1):69–83. DOI: 10.1007/s12307-008-0007-2 [PubMed: 19308686]
101. Spizzo R, Almeida MI, Colombatti A, Calin GA. Long non-coding RNAs and cancer: a new frontier of translational research? Oncogene. 2012; 31(43):4577–87. DOI: 10.1038/onc.2011.621 [PubMed: 22266873]
102. Huang X, Yuan T, Tschannen M, Sun Z, Jacob H, Du M, Liang M, Dittmar RL, Liu Y, Kohli M, Thibodeau SN, Boardman L, Wang L. Characterization of human plasma-derived exosomal RNAs by deep sequencing. BMC Genomics. 2013; 14:319.doi: 10.1186/1471-2164-14-319 [PubMed: 23663360]
103. Prensner JR, Iyer MK, Balbin OA, Dhanasekaran SM, Cao Q, Brenner JC, Laxman B, Asangani IA, Grasso CS, Kominsky HD, Cao X, Jing X, Wang X, Siddiqui J, Wei JT, Robinson D, Iyer HK, Palanisamy N, Maher CA, Chinnaiyan AM. Transcriptome sequencing across a prostate cancer cohort identifies PCAT-1, an unannotated lincRNA implicated in disease progression. Nat Biotechnol. 2011; 29(8):742–9. DOI: 10.1038/nbt.1914 [PubMed: 21804560]

104. Lai MC, Yang Z, Zhou L, Zhu QQ, Xie HY, Zhang F, Wu LM, Chen LM, Zheng SS. Long non-coding RNA MALAT-1 overexpression predicts tumor recurrence of hepatocellular carcinoma after liver transplantation. Med Oncol. 2012; 29(3):1810–6. DOI: 10.1007/s12032-011-0004-z [PubMed: 21678027]

105. Tinzl M, Marberger M, Horvath S, Chypre C. DD3 PCA3 RNA Analysis in Urine–A New Perspective for Detecting Prostate Cancer. European urology. 2004; 46(2):182–187. 2004. DOI: 10.1016/j.eururo.2004.06.004 [PubMed: 15245811]

106. Katrin P, Tschernatsch MM, Guelly C, Moustafa T, Stradner M, Strohmaier HM, Buck CR, Denk H, Schroeder R, Trauner M, Zatloukal K. Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA. Gastroenterology. 2006; 132(1): 330–342. 2007. DOI: 10.1053/j.gastro.2006.08.026 [PubMed: 17241883]

107. Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR, Dahlman JE, Parnas O, Eisenhaure TM, Jovanovic M, Graham DB, Jhunjhunwala S, Heidenreich M, Xavier RJ, Langer R, Anderson DG, Hacohen N, Regev A, Feng G, Sharp PA, Zhang F. CRISPR-Cas9 Knockin Mice for Genome Editing and Cancer Modeling. Cell. 2014; 159(2):440–55. DOI: 10.1016/j.cell. 2014.09.014 [PubMed: 25263330]

108. Han J, Zhang J, Chen L, Shen B, Zhou J, Hu B, Du Y, Tate PH, Huang X, Zhang W. Efficient in vivo deletion of a large imprinted lncRNA by CRISPR/Cas9. RNA Biol. 2014; 11(7):829–35. DOI: 10.4161/rna.29624 [PubMed: 25137067]

109. Niu Y, Shen B, Cui Y, Chen Y, Wang J, Wang L, Kang Y, Zhao X, Si W, Li W, Xiang AP, Zhou J, Guo X, Bi Y, Si C, Hu B, Dong G, Wang H, Zhou Z, Li T, Tan T, Pu X, Wang F, Ji S, Zhou Q, Huang X, Ji W, Sha J. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. Cell. 2014; 156(4):836–43. DOI: 10.1016/j.cell. 2014.01.027 [PubMed: 24486104]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

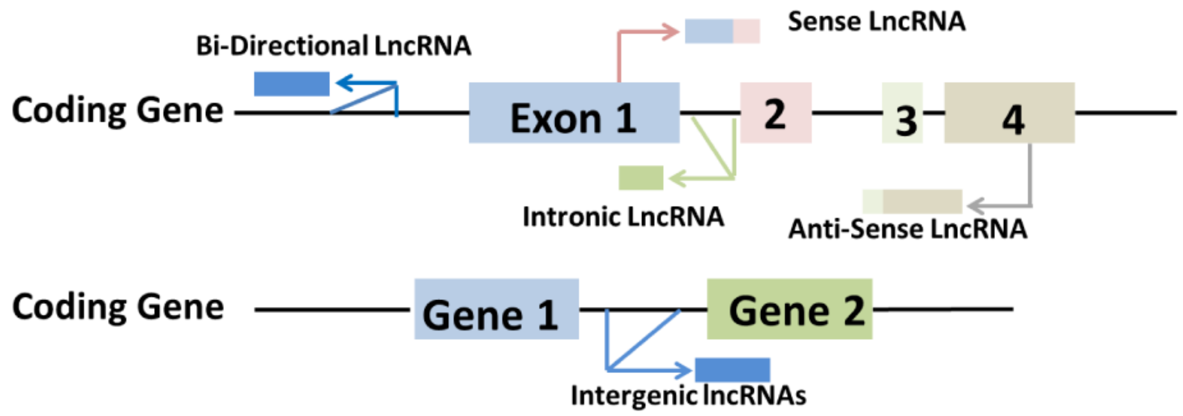

Figure 1.
Five categories of lncRNAs.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

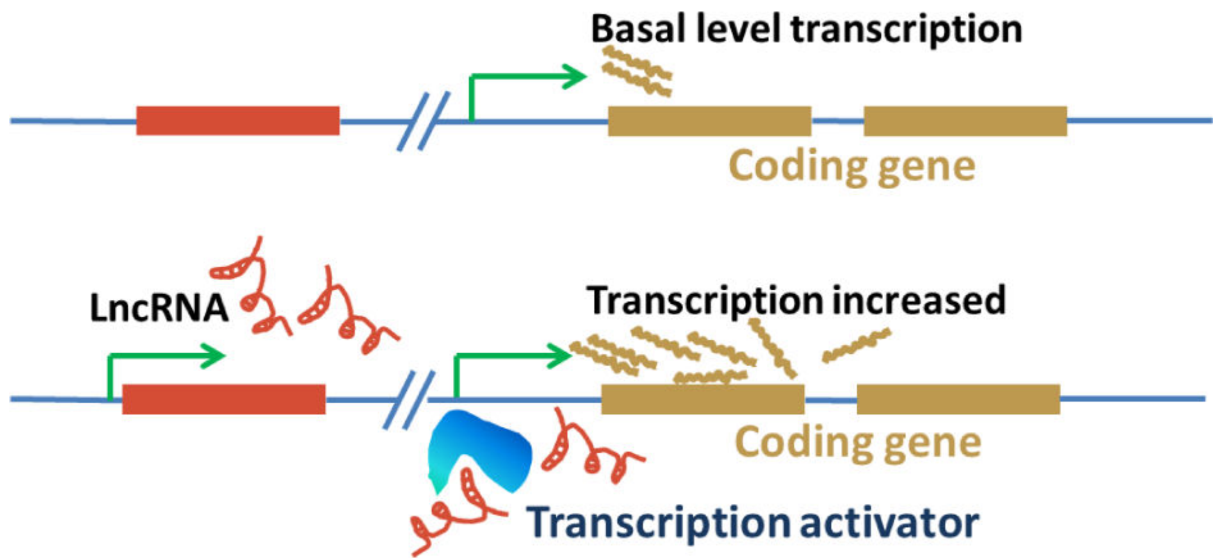

Figure 2. LncRNA enhance transcription

LncRNA transcripts transcribed from ~1Kb upstream of coding gene tethers transcription factors to the promoter of the coding gene which enhances the gene expression.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

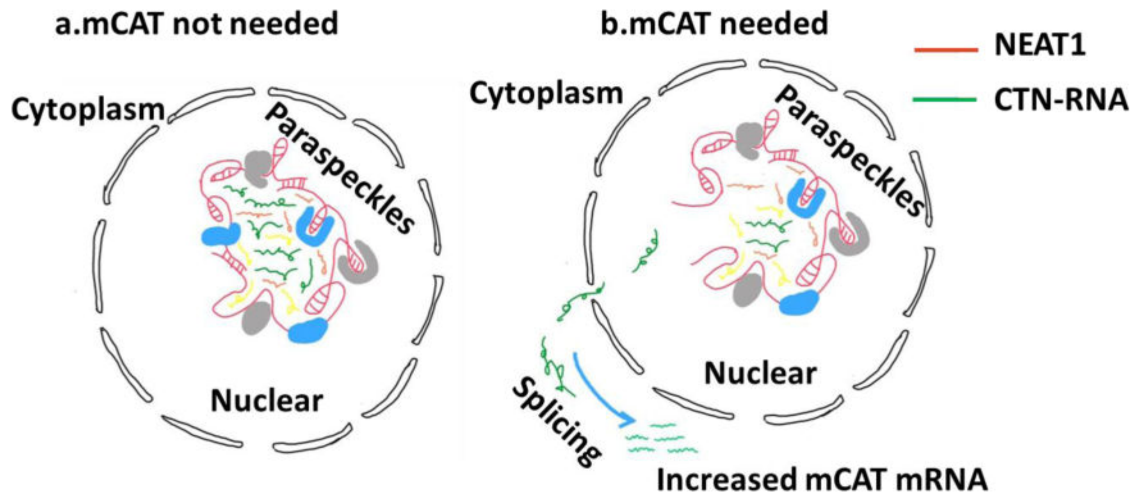

Figure 3. Paraspeckles lncRNA

(a) When cell is not under stress, NEAT1 (red) tether itself and other proteins to form the Paraspeckles which stores RNA in the nuclear including the lncRNA CTN-RNA. (b). When cell is under stress, mCAT is needed. Paraspeckles release the CTN-RNA. CTN-RNA is then transferred to the cytoplasm and spliced to mCAT mRNA followed by increase in mCAT mRNA and protein expression**.**

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Oncogenic and suppressive roles of LncRNAs in breast cancer.

LncRNA name	Function	Mechanism	Reference
Oncogenic			
H19	Gene silencing Promote anchorage-independent growth	Epigenetic	[38,39,40,41]
SRA	Expression activator	Transcriptional	[54,55]
LSINCT5	Promote proliferation	Not known	[57]
Zfas1	Promote proliferation	Not known	[59]
LncRNA-Smad7	Anti-apoptosis	Not known	[60]
LOC554202	Controversy	Not known	[62]
HOTAIR	Suppress invasion and migration	Epigenetic	[68,69,70,71]
SOX2OT	Induce SOX2 expression	Epigenetic	[73]
FAL1	Stabilize BMI1 protein Suppresses p21	Post-transcriptional	[75]
Tumor suppressive			
GAS5	Induce apoptosis	Transcriptional	[79,80,81,82]
XIST	Not known	Epigenetic	[92,93,94]