

LONG NONCODING RNAS AS CANCER BIOMARKERS

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INTRODUCTION: “JUNK DNA,” THE NEW KEY PLAYER IN DEVELOPMENT AND DISEASE

Recent studies have quantified 20,684 human protein-coding genes. These genes comprise just 1.2% of the genome, however, 80% of the genome is functionally transcribed [1]. Therefore it is evident that a significant contribution to the complexity underlying complex organisms derives from nonprotein-coding genes. Until few years ago, the function of the genome in mainstream biology was largely restricted to protein-coding genes and few noncoding RNA species (including ribosomal RNA (rRNA), transfer RNAs (tRNA), and some ribozymes). However, development of the genomic and the transcriptomic technologies have revealed that about 87% of the human genome is actively transcribed and what was more surprising, 70% of those transcripts correspond to noncoding RNA (ncRNA) [2]. These new data led researchers to think that ncDNA was not as “junk” as it was expected before and that it could have a significant functional role in the cell. Several studies have confirmed this initial hypothesis and now ncRNAs are considered legitimate players in development and disease.

Based on their biological functions, ncRNAs are classified into two groups: structural or regulatory ncRNAs. In the first group we can find tRNA, rRNA, small nuclear RNA (snRNA), and small nucleolar RNA (snoRNA). All of them are characterized by being part of the machinery involved in protein synthesis.

The other group is composed of regulatory ncRNAs that depending on their size have also been classified into small ncRNAs (with less than 200 nucleotides in length) such as microRNAs and PIWI-interacting RNAs (piRNA), or those whose transcripts are longer than 200 nucleotides, named long noncoding RNAs (lncRNAs) [3]. All of them are characterized by displaying specific spatiotemporal expression patterns and their importance in most biological processes has increased during the last years. Indeed some authors defend the validity of the RNA world hypothesis that considers RNA not only as a messenger between DNA and proteins but also as a key player in cellular functionality [4].

Thanks to the development of high-resolution and high-throughput techniques such as microarrays and genome wide sequencing, it has allowed the identification and characterization of the noncoding part of our genomes. A recent transcriptome study published in 2015 has identified 58,648 lncRNA genes [5].

It has been proposed to classify lncRNAs into five types based on their genomic location: (1) sense (the lncRNA sequence overlaps with the sense strand of a protein-coding gene), (2) antisense (the sequence overlaps with the antisense strand of a protein-coding gene), (3) bidirectional (the sequence is oriented head-to-head with a protein-coding gene within 1 kilobase), (4) intronic (the sequence is located inside the intron of a protein-coding gene), and (5) intergenic (the sequence is found in a region between two protein-coding genes) [6].

Putting aside the major differences between mRNA and lncRNAs, it has been observed that they share some similarities derived from their transcription process: many lncRNAs are transcribed by RNA polymerase II, their promoters have histone marks that control their regulation, splicing events have also been detected, and these long noncoding transcripts can be polyadenylated and 5'-capped [6]. However, although lncRNAs are expressed at much lower levels than mRNAs, they exhibit more specific tissue expression patterns [7].

Although some ncRNAs, like microRNAs that we previously reviewed [8,9], have a similar way to function due to their common biogenesis, lncRNAs are more heterogeneous due in part to their somewhat arbitrary definition just based on length.

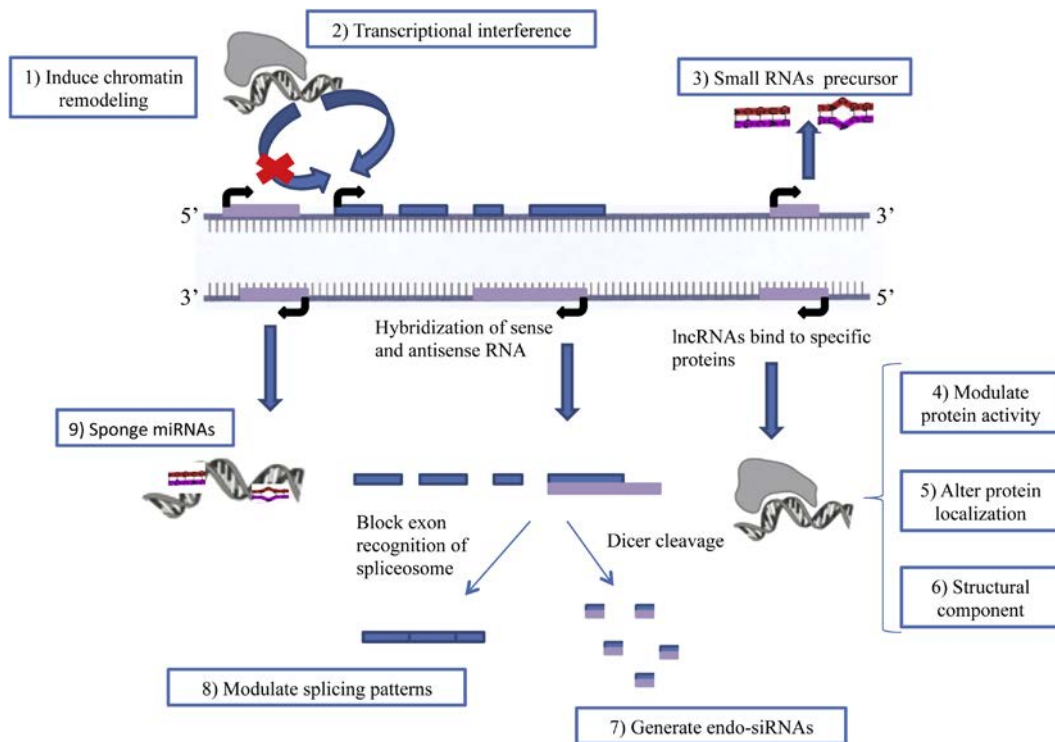
Next, we are going to present some of the most representative functions of lncRNAs:

- 1. Chromatin structure regulation:** lncRNAs can interact with chromatin remodeling complexes and presumably contribute to model chromatin and modify gene expression regulation [10–13].
- 2. Direct transcription regulation:** There are different mechanisms by which lncRNAs are seen to be regulating the transcriptional process. They can interfere with the promoters of protein-coding genes by themselves [14] or by mediating demethylation of those promoters [15]. They are also able to intervene as coactivators of certain transcription factors [16] or, on the contrary, act as decoys of transcription factors, blocking their binding to DNA [17,18]. In addition, it has been observed that the lncRNA MALAT1 (Metastasis Associated Lung Adenocarcinoma Transcript 1) interacts with a type of splicing factor, contributing to the regulation of the mRNA splicing process [19].
- 3. Modulators of mRNA stability:** lncRNAs can regulate the stability of their target mRNAs by activating the SMD (Staufen 1-Mediated messenger RNA Decay) process that is involved in degrading mRNAs [20]. Other studies have also shown that other lncRNAs can increase the stability of mRNAs by binding to them and blocking their degradation [21].
- 4. mRNA translation regulation:** It has been observed that some lncRNAs can decrease the translation of transcripts by reducing polysomes or inducing what is known as ribosome “drop-off” [22]. On the other hand, other lncRNAs promote the translational process by overlapping at the 5'-end of their target mRNA and improving the association of polysomes–mRNA [23].
- 5. Contributors to the organization of different nuclear structures:** Some lncRNAs are also involved in the maintenance of the nuclear architecture across chromosomes such as the long intergenic noncoding RNA (lincRNA) Firre (Functional Intergenic Repeating RNA Element) [24].
- 6. Regulators of other noncoding transcripts:** It has been observed that some lncRNAs act as precursors of other noncoding transcripts that have less than 200 nucleotides such as microRNAs [25–27]. lncRNAs can also function as molecular decoys or sponges of microRNAs affecting their free levels and therefore the function of those microRNAs. This type of lncRNAs is known as competing endogenous RNA (ceRNA) [28]. In addition, lncRNAs can also compete with microRNAs for their binding to their target mRNAs [29].
- 7. Protein activity regulation:** There is a special subtype of lncRNAs that is able to regulate the activity of certain proteins. This subtype is termed sno-lncRNAs because their sequence is flanked by other sequences that codify for snoRNA. Some studies have discovered that several members of sno-lncRNAs can bind to an alternative splicing factor regulating its activity and therefore interfering with the splicing process of the cell [30] (Fig. 6.1).

These are just some examples of a growing list that reveal the vast number of processes in which lncRNAs can be involved and that are burying the concept of “junk” noncoding DNA.

LONG NONCODING RNAs AS CANCER BIOMARKERS ROLE IN TUMORIGENESIS

lncRNAs offer appealing characteristics as cancer biomarkers due to the following observations: (1) Some lncRNAs have high specificity and sensitivity to the tumoral process; (2) Some lncRNA can be sampled in body fluids with relative stability; (3) Some lncRNAs are useful as diagnostic and/or

**FIGURE 6.1**

Representation of some cellular functions of long noncoding RNAs (lncRNAs).

Adapted from Shi X. et al. Cancer Lett 2013.

prognostic tools in oncology since they behave as predictors of survival time, risk of metastasis, and recurrence.

Next, we are going to discuss in detail all these characteristics:

- 1. Some lncRNA have high specificity and sensitivity to the tumoral process.** Since lncRNAs are effector molecules, their expression levels, which are more tissue-specific compared with protein-coding genes [7], are good indicators of the tumor state. As it has been described in the previous part of this chapter, the functionality of lncRNAs can contribute to cell homeostasis and therefore their unbalance can lead to a pathological status.

The contribution of lncRNAs has been specially reported in several pathologies, including cancer where several lncRNAs have been found differentially expressed in tumors in comparison with normal tissue. It has been observed that lncRNAs can act either as oncogenes when they promote the tumor development or as tumor suppressors when they inhibit tumor development [31]. Indeed, some lncRNAs have different functions to play a significant role in cancer, which make their expression levels be specific and sensitive to the tumoral process. For example, due to the role of lncRNAs in gene expression, it is of no surprise to find that protein-coding **oncogenes**

and tumor suppressor genes are also targets of some lncRNAs [32,33]. For instance, if we focus on one of the most studied tumor suppressors, p53, it has been discovered that a great number of lncRNAs that are related to its regulation. MEG3 (Maternally Expressed Gene 3 imprinted lncRNA) is able to activate p53 in lung cancer [34] and meningioma [35] controlling therefore apoptosis and proliferation. Other lncRNA involved in p53 pathway is lincRNA-p21. In this case the expression of the lncRNA is regulated by p53, but it has been seen in mice that lincRNA-p21 promotes transcription of p21, an important G1/S checkpoint, leading to an increment in proliferation [36,37].

Some lncRNAs have a significant role in **chromatin structure**. As matter of fact, several chromatin remodeling complexes such as PRC1 and 2 or SWI/SNF are seen to be modulated by lncRNAs [38,39]. For example, SchLAPI (Second Chromosome Locus Associated with Prostate-1) is a lncRNA highly upregulated in prostate cancer (PCa), which is able to bind to SWI/SNF complex and impede its interaction with the genome. As a result, this lncRNA is capable of promoting tumor invasion and metastasis [13]. However, not all lncRNAs require chromatin remodeling complexes to exert their effect. The lncRNA TARID (TCF21 Antisense RNA Inducing Demethylation) recognizes the promoter of the tumor-suppressive transcription factor TCF21 and promotes its demethylation by recruiting a DNA demethylator (GADD45A). TARID has been found to be inactivated by promoter hypermethylation in cancer leading to a blockage in the expression of tumor suppressor TCF21 [15].

In addition, there are lncRNAs that play a significant **role in cell-signaling pathways** that are key for the maintenance of cellular homeostasis and the prevention of carcinogenesis [40]. Only to mention a few examples, it has been seen that lncRNA-ATB (lncRNA-activated by TGF- β) is induced after stimulation with TGF- β promoting IL-11 stabilization and therefore contributing to cancer cell metastasis in hepatocellular carcinoma (HCC) [41]. In other cases, hormone receptors are able to modulate lncRNA expression. For instance, NEAT1 (Nuclear Enriched Abundant Transcript 1) is a lncRNA that is regulated by estrogen receptor α (ER- α) and is highly upregulated in PCa. Indeed this lncRNA is able to interact with promoters of the genes involved in PCa contributing therefore to tumorigenesis [42]. Other lncRNAs are seen to act as mediators in the signaling pathways. NKILA (NF- κ B interacting lncRNA) is a lncRNA whose expression is upregulated by NF- κ B and is involved in a negative feedback loop that controls NF- κ B activation. NKILA stabilizes NF- κ B/I κ B complex, protecting I κ B from degradation and therefore impeding the subsequent activation of NF- κ B. In breast cancer, low levels of NKILA are associated with metastasis and larger tumor sizes decreasing patient survival [43].

2. Some lncRNAs can be sampled in body fluids with relative stability

Another important fact that has increased the interest of the scientific community on lncRNAs is that they are not only deregulated at their intracellular levels. It has been observed that the alteration of lncRNAs levels is also **detected in extracellular body fluids**, which are more accessible to sample patients. This is a very relevant point to take in mind because one of the most important characteristics that should have a good biomarker is an easy accessibility that enables noninvasive techniques to collect samples.

In addition, it has been confirmed that circulating nucleic acids (CNAs), either DNA or RNA molecules, maintain the changes associated with tumorigenesis so they are reliable sources for cancer diagnosis and prognosis [43].

The mechanisms of lncRNA secretion into body fluids are currently under study. It is thought that their secretion is mediated by exosomes or microvesicles formation. These are lipoprotein vesicles with 50–100 nm of diameter that are formed through the exocytic process. Different studies have revealed that not only lncRNAs are present in a 3.36% of all exosomal RNA sequences [44], but also that the levels of lncRNAs in exosomes and plasma are very similar. This fact suggests that the majority of plasmatic lncRNAs are inside exosomes [45]. Additionally, some authors have described that cells from the tumor or adjacent to it are able to release lncRNAs that can enter directly to circulation [30,45,46].

3. **Some lncRNAs are useful as diagnosis and/or prognosis tools** in oncology and behave as predictors of survival time, risk of metastasis, and recurrence [33]. Due to the great number of biological processes in which lncRNAs are involved, these molecules reflect the dynamical status of the tumor and shed light on the evolution of the cancer in patients.

In the following parts of this chapter some of the most validated lncRNAs biomarkers are mentioned and divided into diagnostic or prognostic tools for cancer patients.

POTENTIAL USAGE AS DIAGNOSTIC AND PROGNOSTIC BIOMARKERS OF DIFFERENT TUMORS

Despite the advances in diagnostic procedures, nowadays it is difficult to find a method that can effectively diagnose cancer patients. Only to mention one example of this fact are NSCLC (non-small cell lung cancer) patients. 40% of them are diagnosed with high-grade tumors or with metastasis, hindering their treatment and therefore their survival [45]. So there is an urgent need of developing new methods of diagnosis that enable early detections and less overdiagnosis and overtreatments.

To date, the screening of biomarkers in extracellular fluids from the patient is one of the most promising methods for early diagnosis as well as the most noninvasive technique.

As it has been previously explained, lncRNAs are part of a group of molecules called CNAs and also their levels are good markers of the tumor state since an unbalance in their expression leads to loss of cellular homeostasis.

Several studies have been done to detect the utility of lncRNAs as biomarkers not only as diagnostic tools but also in prognosis. Given that lncRNAs are involved in tumorigenesis, tumor progression, invasion, and metastasis, it is of no surprise that these molecules can also provide useful information about the dynamical status of the tumor.

Next, we have compiled some of the most significant findings in different tumors types.

Prostate Cancer

Nowadays, the diagnostic method for PCa commonly used in the clinic is the detection of the androgen-regulated serine protease PSA (prostate-specific antigen) in serum. The expression of this protein is specific to prostate tissue and its levels are increased in PCa. However, it has been observed that PSA levels in serum are not so specific to this type of cancer. They are also increased in benign prostatic hyperplasia and prostatitis [46]. So this diagnostic method can lead to overdiagnosis and overtreatment. This approach has better results as a prognosis biomarker [47].

Several authors have shown that lncRNAs can be more specific biomarkers to perform more accurate diagnosis of PCa. PCA3 (Prostate Cancer Antigen 3, also known as DD3) is a lncRNA

whose expression is increased in more than 95% of primary PCa tumors [48], and it can be detected in urine samples from patients. Indeed a urine-based molecular diagnostic test known as *PROGENSA PCA3 test* has been developed, which was approved in 2012 by the Food and Drug Administration (FDA) [48–50]. The validity of this PCa biomarker for PCa was confirmed by performing a meta-analysis using different studies. The results were published in 2014 and assigned PCA3 a sensitivity of 62% and a specificity of 75% in PCa [51]. These data together with the fact that PCA3 levels in urine also correlate with tumor aggressiveness [52] make this lncRNA biomarker a promising candidate for PCa prognosis too [53]. However, some technique limitations have also been described such as significant intraindividual variability and low sensitivity [54,55]. For this reason, the latest studies have shown that a combination of biomarkers including PCA3 as well as PSA could overcome this drawback [56].

However, this lncRNA is not the only one that can be found altered in PCa. MALAT1 is another lncRNA that has been found in plasma at higher levels in patients with this type of tumor. With a sensitivity of 59% and a specificity of 85%, MALAT1 is also considered as a diagnostic biomarker for PCa [57]. In addition, other studies have revealed that MALAT1 could be detected not only in blood but also in urine. It has been observed that MALAT1 levels are higher in urine of patients with positive biopsies suggesting the potential of this lncRNA as urine diagnostic biomarker for PCa [58]. The authors of this study also highlight the utility that this lncRNA could avoid between 30.2% and 46.5% of biopsies in patients who have a PSA concentration between 4 and 10 ng/mL in serum.

lncRNA-PCAT-18 (Prostate Cancer-Associated Noncoding RNA Transcript 18) is another lncRNA recently discovered by RNA sequencing that is specifically expressed in prostate tissue and whose levels are higher in PCa in comparison with other benign and tumoral samples. This lncRNA can be detected in plasma and also can give information about the metastatic status of the tumor since its expression is higher in metastatic PCa than in localized PCa [59].

With regard to potential prognostic lncRNA biomarkers, SchLAP1 could be one of them. The expression of SchLAP1 is increased in 25% of PCa patients, and it is associated with risk of recurrence and mortality after prostatectomy [13]. Three independent cohorts have confirmed that SchLAP1 levels in urine sediments can be used to distinguish between patients with low or high risk of recurrence [60].

Lung Cancer

To date, most of the biomarkers that are used for NSCLC diagnosis are proteins that are released by tumor cells such as chromogranin, CEA (Carcinoembryonic Antigen), NSE (neuron-specific enolase), CA125 (Carcinoma Antigen 125, also known as mucin 16), CA19-9 (Carbohydrate Antigen 19-9), and CYFRA21-1 (Cytokeratin 19 Fragment) [61,62]. However, although these biomarkers are very sensitive, they could be better to avoid aberrant or late diagnosis [63].

This is the reason why other alternatives for NSCLC diagnosis are also being searched.

MALAT1 was one of the first lncRNAs found associated with cancer, but until some years ago it was not confirmed its potential as diagnostic biomarker for NSCLC. From a cohort of 105 lung cancer patients and 65 healthy controls, it was seen that MALAT1 levels in blood samples were lower in lung cancer patients than in controls, contrary to what can be observed in tissue [64]. This lncRNA can give a specificity of 96% but, due to its low sensitivity (56%), MALAT1 should be measured with other biomarkers to improve the diagnosis [65].

In 2015 another report found that up to 64 lncRNAs were deregulated in NSCLC tumor samples in comparison with adjacent normal lung tissue. In addition, they detected 181 lncRNAs that were

differentially expressed depending on the histological subtype of NSCLC [66]. Also recently, a 5-lncRNA panel using microarray data has been developed, which is able to detect early-stage lung adenocarcinoma with high sensitivity and specificity [67]. Additionally, there have been other publications that emphasize the property of lncRNAs as fingerprints of NSCLC that can be easily traced in patient's body fluids. In this case, the authors mention three lncRNAs (SPRY4-IT1, Antisense Noncoding RNA in the INK4 Locus (ANRIL), and NEAT1) that are able to diagnose NSCLC patients with a sensitivity of 82.8% and a specificity of 92.3% using plasma samples. In addition, the expression of these lncRNAs is associated with tumor size therefore providing information about tumor burden [68].

There are also three lncRNAs that could be applied to lung cancer prognosis:

Like in other types of tumors, HOTAIR (HOX transcript antisense intergenic RNA) is a lncRNA whose expression is associated with poor survival and it is seen as a possible prognostic biomarker for NSCLC [69].

BANCR (BRAF-regulated lncRNA 1) is other lncRNA that promotes cell migration and invasion in NSCLC by regulating essential proteins involved in EMT (epithelial mesenchymal transition). For this reason, some studies suggest this lncRNA as a measure of the metastatic status of NSCLC [70].

CARLo-5 (Cancer-Associated Region Long noncoding RNA) is also described as a lncRNA with a negative prognostic ability for NSCLC [71].

Breast Cancer

As in other types of tumors, serum biomarkers such as CEA and CA125 display clinical utility for breast cancer diagnosis. However, they have a limited specificity since they are also used for the detection of other cancers [72].

A study performed with 45 patients with breast cancer has revealed that H19, HOTAIR, and KCNQ1OT1 (KCNQ1 Opposite Strand/Antisense Transcript 1) are some lncRNAs that could be used as diagnostic biomarkers using a technique known as chromogenic in situ hybridization (CISH) [73].

Other authors have shown the utility of RP11-445H22.4 as a breast cancer biomarker. RP11-445H22.4 is highly expressed in breast cancer tissues and in serum samples of patients in comparison with healthy controls with a sensitivity and specificity of 92% and 74%, respectively [74]. However, these are only preliminary results that should be validated with large-scale studies.

With regard to prognosis, it has been observed that HOTAIR levels are correlated with the survival rate of breast cancer patients. High levels of HOTAIR expression are associated with reduced survival and an increase of metastasis [10].

BCAR4 (Breast Cancer Antiestrogen Resistance 4) also has a promising potential as a prognostic biomarker for breast cancer. BCAR4 is highly upregulated in later-stage tumors and with metastasis, and it is associated with shorter survival rates in breast cancer patients [75].

NKILA is other lncRNA correlated with metastasis, advanced stage tumors, and shorter survival rates in breast cancer patients NKILA [43].

Hepatocellular Carcinoma

In HCC, it has been observed that the lncRNA HULC (high upregulated in liver cancer) is upregulated in plasma from patients. However, there is no information about its sensitivity and specificity [76]. What is seen is that HULC has better possibilities to discriminate between HCC and controls in combination with the lncRNA LINC00152 [77].

Another study used a panel of three lncRNAs (RP11-160H22.5, XLOC_014172, and LOC149086) for HCC diagnosis with plasma samples. With this approach, a sensitivity of 82% and a specificity of 73% were obtained [78]. In addition, these authors also emphasize the prognostic potential of XLOC_014172 and LOC149086 since they can distinguish between patients with or without metastasis.

Also recently, lncRNA-AF085935 was found as promising HCC diagnostic biomarker since it can be detected in serum and discriminate among HCC patients, hepatitis B–infected patients, and healthy controls [79].

In addition, three different studies have revealed the potential of certain lncRNAs to be applied in HCC prognosis.

High levels of HOTAIR are associated with poor prognosis of HCC patients since it is correlated with the risk of recurrence and lymph node metastasis [80].

MALAT1 is other lncRNA that can be used to predict tumor recurrence after liver transplantation [81].

HOTTIP (HOXA transcript at the distal tip) can also be considered as other prognostic biomarker for HCC. It is upregulated in HCC, and some researchers have showed that its expression is correlated with clinical progression of HCC and survival [82].

Bladder Carcinoma

To date there are some studies that highlight the potential of linc-RNA UCA1 (Urothelial Carcinoma Associated 1) as a lncRNA that can be applied for bladder cancer diagnosis. UCA1 can be detected in the cellular sediment of patients' urine and can distinguish between bladder carcinoma and other urinary diseases with a sensitivity of 80.9% and a specificity of 91.8% [83,84].

Not only UCA1 could act as a diagnostic biomarker but also in prognosis. It has been observed that blood levels of UCA1 are higher in those patients that have advanced tumors after cisplatin-based combination chemotherapy. For this reason, UCA1 has been proposed as a biomarker to monitor the outcome of chemotherapy in this type of cancer [85].

Gastric Cancer

Alternatives to protein-coding biomarkers have also been searched to diagnose gastric cancer (GC) patients. One of them is the lncRNA H19 whose expression is increased in tumor tissues in comparison with adjacent normal tissue. Some authors suggest that H19 could be used as a diagnostic biomarker for GC in plasma samples. The levels of this lncRNA are higher in GC patients than in controls with a sensitivity and specificity of 82.9% and 72.9%, respectively [86]. In addition, it was observed that the levels of H19 in plasma are correlated with cancer tissue size since H19 expression was reduced in postoperative samples.

Another lncRNA that has been found altered in plasma samples of GC patients is LINC00152. Some researchers suggest that this lncRNA could also be used for GC diagnosis with a sensitivity of 48.1% and a specificity of 85.2% [87].

Other report has used a combination of three lncRNAs: UCA1, LSINCT-5, and PTENP1 to improve the GC diagnosis. With this panel of lncRNA they were able to detect early GC with a specificity and a sensitivity of 97% and 77.8%, respectively, as well as discriminate between patients with benign peptic ulcers and GC patients with a sensitivity of 91.7% and a specificity of 83.3% [88].

But blood is not the only body fluid that can be analyzed from patients. Gastric juice is another source of information that can be used in GC diagnosis. AA174084 is a lncRNA whose levels are increased in gastric juice from GC patients in comparison with healthy controls or individuals with other gastric mucosa lesions. Although the sensitivity of this potential biomarker is only 46%, the specificity is very high: 93% [89].

UCA1, a lncRNA mentioned previously as a biomarker in bladder cancer diagnosis, has also been observed and overrepresented in gastric juice from GC patients in comparison with healthy individuals [90].

With regard to prognosis, it has been reported that ANRIL levels correlate with poor prognosis. This is a lncRNA that has an important role in tumor progression and whose expression is highly upregulated in GC [91].

Other possible biomarker of worse prognosis is GAS5 (Growth Arrest-Specific 5). It is a tumor suppressor lncRNA that when is downregulated promotes tumor proliferation. Therefore, low expression of GAS5 is associated with poor outcomes in GC [92].

Colorectal Cancer

Although there are different diagnostic tests such as fecal occult-blood testing, colonoscopy, and stool DNA testing, these techniques have a limited potential since their sensitivity and reproducibility are very low.

However, a study has revealed that there are three lncRNA (XLOC_006844, LOC152578, and XLOC_000303) that could be used as a panel for colorectal cancer (CRC) diagnosis. Their plasmatic levels are increased in CRC patients in comparison with healthy controls [93].

HULC is another lncRNA that not only is upregulated in HCC but also in CRC. However, this lncRNA is only detected in those CRC that have hepatic metastasis, not in primary colon tumors or with metastasis in other tissues [94].

In addition, there are some lncRNAs that could be used in clinic for CRC prognosis. One of them is HOTAIR. As with other types of cancer, this lncRNA is associated with poor prognosis, and in this case it also correlates with liver metastasis [95].

MALAT1 is another lncRNA that is highly expressed in CRC patients with poor postoperative prognosis [96].

Oral Squamous Cell Cancer

A recent study showed that the lncRNAs HOTAIR and MALAT1 are found overrepresented in oral squamous cell cancer (OSCC) patients' saliva in comparison with control samples and they can be easily measured by qPCR [97]. Interestingly, it was also seen that patients with lymph node metastasis had higher levels of HOTAIR in their saliva.

Esophageal Squamous Cell Carcinoma

Protein-coding biomarkers such as SCCA (squamous cell carcinoma antigen), CA19-9, and CEA have been validated for esophageal squamous cell carcinoma (ESCC) diagnosis [62], but other biomarkers (including lncRNAs) are being analyzed to improve the diagnosis sensitivity and specificity.

A recent study published in 2015 showed that plasma levels of three lncRNAs: POU3F3, HNF1A-AS1, and SPRY4-IT1 could detect early-stage ESCC with a sensitivity and a specificity of 85.7% and 81.4%, respectively [98].

B-Acute Lymphoblastic Leukemia

A recent report has found that there are lncRNAs that are able to predict the cytogenetic subtype of B-acute lymphoblastic leukemia (B-ALL) among the three most common abnormalities: mixed lineage leukemia (MLL) rearrangement, TEL-AML1 fusion, and E2A-PBX fusion. This specific subset of lncRNAs is called B-ALL-associated long RNAs (BALR).

High expression levels of BALR-2 are associated with poorer responses to prednisone treatment. These results support the current hypothesis that BALR-2 can cause resistance to apoptosis [99].

The number of different types of tumors is very high as well as the number of studies that are currently focused on discovering new lncRNA that could be applied to cancer diagnosis and prognosis. The lncRNAs mentioned are only some examples that reflect their future potential (Table 6.1).

Table 6.1 Compilation of Long Noncoding RNAs (lncRNAs) With Biomarker Potential in Cancer

Cancer Type	lncRNA(s)	Up/Down	Diagnosis	Prognosis	References
Prostate	PCA3	Up	Yes	Yes	[48–56]
Prostate	MALAT1	Up	Yes	–	[57,58]
Prostate	PCAT-18	Up	Yes	–	[59]
Prostate	SChLAP1	Up	–	Yes	[13,60]
Lung	MALAT1	Down	Yes	–	[64,65]
Lung	5-lncRNA panel	1up/4down	Yes	–	[67]
Lung	SPRY4-IT1, ANRIL, NEAT1	Up	Yes	–	[68]
Lung	HOTAIR	Up	–	Yes	[69]
Lung	BANCR	Up	–	Yes	[70]
Lung	CARLo-5	Up	–	Yes	[71]
Breast	H19, HOTAIR, KCNQ1OT1	Up	Yes	–	[73]
Breast	RP11-445H22.4	Up	Yes	–	[74]
Breast	HOTAIR	Up	–	Yes	[10]
Breast	BCAR4	Up	–	Yes	[75]
Breast	NKILA	Down	–	Yes	[43]
Liver	HULC	Up	Yes	–	[76,77]
Liver	RP11-160H22.5, XLOC_014172, LOC149086	Up	Yes	–	[78]
Liver	lncRNA-AF085935	Up	Yes	–	[79]
Liver	HOTAIR	Up	–	Yes	[80]
Liver	MALAT1	Up	–	Yes	[81]
Liver	HOTTIP	Up	–	Yes	[82]
Bladder	UCA1	Up	Yes	Yes	[83–85]
Stomach	H19	Up	Yes	–	[86]
Stomach	LINC00152	Up	Yes	–	[87]

Continued

Table 6.1 Compilation of Long Noncoding RNAs (lncRNAs) With Biomarker Potential in Cancer—cont'd

Cancer Type	lncRNA(s)	Up/Down	Diagnosis	Prognosis	References
Stomach	UCA1, LSINCT-5, PTENP1	Down	Yes	–	[88]
Stomach	AA174084	Up	Yes	–	[89]
Stomach	UCA1	Up	Yes	–	[90]
Stomach	ANRIL	Up	–	Yes	[91]
Stomach	GAS5	Down	–	Yes	[92]
Colon	XLOC_006844, LOC152578, XLOC_000303	Up	Yes	–	[93]
Colon	HULC	Up	Yes	–	[94]
Colon	HOTAIR	Up	–	Yes	[95]
Colon	MALAT1	Up	–	Yes	[96]
Oral	HOTAIR, MALAT1	Up	Yes	–	[97]
Esophagus	POU3F3, HNF1A-AS1, SPRY4-IT1	Up	Yes	–	[98]
B Cells	BALR-2	Up	–	Yes	[99]

CONCLUSIONS AND FUTURE PERSPECTIVES

During the last decade the field of ncRNAs has increased in popularity and importance in the scientific community. Nowadays, the role of lncRNAs in tumor biology as well as in other diseases is undeniable. Several studies have proposed them as important players that should be evaluated to gain information about the tumor identity as well as its dynamical status.

For this reason, linked to the lack of sensitive and specific diagnostic or prognostic cancer biomarkers, lncRNAs have emerged as a promising tool to be applied in clinic in the near future.

In this chapter, we have mentioned different lncRNAs that have been found altered in cancer and how their levels are able to correlate with distinct tumor phenotypes, without pretension to completeness considering the rapid growth of this field.

In addition, lncRNAs offer several advantages that make them more suitable for the development of diagnostic and prognostic tools in cancer. The expression of some lncRNAs is sensitive to a pathological state and is also considerably stable in body fluids enabling the development of noninvasive techniques.

However, the field of lncRNAs as cancer biomarkers is still in a very preliminary stage, and there are several inconsistencies among the studies that have been published to date. To promote the usage of circulating lncRNAs in clinic, it is needed building a standardization in the procedures followed by researchers when analyzing these potential biomarkers. It has been proposed that four points should be considered to ensure that changes in lncRNAs properly reflect the pathology [33]:

1. Standardization of sample collection, extraction, and storage methods.
2. Set endogenous controls for lncRNA expression normalization.

3. Define consensus methods for determining the quality of the results obtained.
4. Increase the cohort size to minimize interindividual variability.

In addition, there is a new field that is becoming increasingly important. The findings of mutations, amplifications, or deletions in lncRNA sequences in cancer are making researchers wonder how these gene variations could affect the lncRNA structure and therefore its function. This information could also be used to study patients at the genomic level to see if they have these alterations that can make them prone to a certain type of cancer or that can give them poorer survival outcomes.

All things considered, although there are still some limitations to overcome, it can be claimed that lncRNAs are very promising tools for oncologist to all levels: diagnosis, prognosis, and even in therapy.

LIST OF ACRONYMS AND ABBREVIATIONS

ANRIL	Antisense Noncoding RNA in the INK4 Locus
B-ALL	B-acute lymphoblastic leukemia
BALR	B-ALL-associated long RNAs
BANCR	BRAF-regulated lncRNA 1
BCAR4	Breast Cancer Antiestrogen Resistance 4
BRAF	B-Raf proto-oncogene, serine/threonine kinase
CA125	Carcinoma Antigen 125, also known as mucin 16
CA19-9	Carbohydrate Antigen 19-9
CARLo-5	Cancer-Associated Region Long noncoding RNA
CEA	Carcinoembryonic Antigen
ceRNA	Competing endogenous RNA
CISH	Chromogenic in situ hybridization
CNAs	Circulating nucleic acids
CRC	Colorectal cancer
CYFRA21-1	Cytokeratin 19 Fragment
EMT	Epithelial mesenchymal transition
ENCODE	Encyclopedia of DNA Elements
ER-α	Estrogen receptor α
ESCC	Esophageal squamous cell carcinoma
FIRRE	Functional intergenic repeating RNA element
GAS5	Growth Arrest-Specific 5 lncRNA
GC	Gastric cancer
H19	Imprinted maternally expressed transcript
HCC	Hepatocellular carcinoma
HNF1A	Hepatic Nuclear Factor 1 Homeobox A
HNF1A-AS1	HNF1A Antisense 1
HOTAIR	HOX transcript antisense intergenic RNA
HOTTIP	HOXA transcript at the distal tip
HOX	Homeobox
HULC	High upregulated in liver cancer
IL-11	Interleukin 11
INK4	CDKN2A: Cyclin-Dependent Kinase Inhibitor 2A
IκB	Inhibitor of kappa B

KCNQ1	Potassium Voltage-Gated Channel Subfamily Q Member 1
KCNQ1OT1	KCNQ1 Opposite Strand/Antisense Transcript 1
lncRNA	Long noncoding RNA
lncRNA-ATB	lncRNA-activated by TGF- β
LSINCT5	Long Stress-Induced Noncoding Transcript 5
MALAT1	Metastasis Associated Lung Adenocarcinoma Transcript 1
MEG3	Maternally Expressed Gene 3 imprinted lncRNA
MLL	Mixed lineage leukemia
mRNA	Messenger RNA
ncRNA	Noncoding RNA
NEAT1	Nuclear enriched abundant transcript 1
NF-κB	Nuclear factor kappa B
NKILA	NF- κ B interacting lncRNA
NSCLC	Non-small cell lung cancer
NSE	Neuron-specific enolase
OSCC	Oral squamous cell cancer
PCa	Prostate cancer
PCA3	Prostate Cancer Antigen 3
PCAT-18	Prostate Cancer-Associated Noncoding RNA Transcript 18
piRNA	PIWI-interacting RNA
POU3F3	POU Class 3 Homeobox 3
PRC2	Polycomb Repressive Complex 2
PSA	Prostate-specific antigen
PTENP1	Phosphatase and Tensin Homolog Pseudogene 1
rRNA	Ribosomal RNA
SCCA	Squamous cell carcinoma antigen
SChLAP1	Second Chromosome Locus Associated with Prostate-1
SMD	Staufen 1-Mediated messenger RNA Decay
snoRNA	Small nucleolar RNA
snRNA	Small nuclear RNA
SPRY4	Sprouty RTK signaling antagonist 4
SPRY4-IT1	SPRY4 Intronic Transcript 1
SWI/SNF	SWItch/Sucrose nonfermentable
TARID	TCF21 Antisense RNA Inducing Demethylation
TCF21	Transcription Factor 21
TGF-β	Transforming growth factor beta
tRNA	transfer RNA
UCA1	Urothelial Carcinoma Associated 1 lncRNA

GLOSSARY

Biomarker The International Programme on Chemical Safety, led by the World Health Organization (WHO) and in coordination with the United Nations and the International Labor Organization, defines a biomarker as “any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease.”

Diagnosis Process by which physicians can explain patients’ symptoms and signs. They study their background and the results obtained after the tests and determine which is the most probable disease or disorder that can be affecting those patients.

lncRNA Long noncoding RNAs are a heterogeneous group of RNA molecules with more than 200 nucleotides of length that do not encode proteins, or at least more than 100 amino acids. lncRNAs comprise the major proportion of noncoding transcripts, and they are involved in several cellular and developmental processes. Moreover, they have strong spatiotemporal expression patterns, and their deregulation is associated with different pathological status. Due to these characteristics, lncRNAs have emerged as important molecules that can be applied in clinic for treatment, diagnosis, or prognosis of certain diseases.

Prognosis This term can be defined as the hypothesis of the evolution of a certain disease. The prospect of recovery or the symptoms that can appear during the time are some data that can be predicted with the appropriate prognostic tools.

Tumorigenesis Pathological process where normal cells acquire an unlimited proliferative capacity and an insensitivity to antigrowth signals. This process is not a unique step but several reactions that usually start with genotypical changes and end with phenotypical alterations that will produce malignant cells.

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