

Long Non-Coding RNAs: Novel Emergent Biomarkers for Cancer Diagnostics

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Abstract Long non-coding RNAs are a novel class of approximately 15,000 – 20,000 non-protein coding RNAs, having a base length of over 200 nucleotides and are the result of RNA Polymerase II activities. Long non-coding RNAs have only just recently been identified to play a major role in gene regulatory pathways for a wide spectrum of human disease conditions, including multiple cancer models. This review article serves to outline, through recent scientific evidence, the emerging importance of long non-coding RNAs as key biomarkers in cancer diagnostics and therapeutics within the clinical setting. Ultimately, there will be soon the establishment of long non-coding RNA expression profile signature analyses on a par level with other molecular diagnostic techniques such as mRNA and miRNA expression profiles for the identification and monitoring of tumour progression and sensitivity to treatment within the individual cancer patient.

Keywords: *lncRNA, miRNA, cancer, biomarker, diagnosis*

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1. Introduction

The discovery and widespread emphasis on RNA interference (RNAi)-based research and development in the past fifteen years has undoubtedly demonstrated that non-coding RNA families have essential roles in gene regulatory pathways and, ultimately, can affect the course of human disease conditions. Such key molecular roles include epigenetic mechanisms in various cancer models [1]. This brief review serves to highlight the main functional roles of the long non-coding RNA (lncRNA) family, particularly regarding gene regulatory activities, and will also describe lncRNAs which have been identified as reliable clinical biomarkers for a wide spectrum of cancer conditions.

2. Description & Biogenesis of LncRNAs

Initial reports regarding the existence of lncRNAs stem from large complementary DNA sequencing feats such as the FANTOM (Functional ANnotation Of Mammalian cDNA) project [2]. The lncRNA family of non-protein coding RNAs is the largest, with over annotated 21,488 lncRNA transcripts in 2012 [3]. The main defining features for lncRNAs are a base length of over 200 nucleotides and the absence of an open reading frame [4,5]. The majority of all transcribed lncRNAs are the result of RNA Polymerase II activity, although it was also

demonstrated that lncRNAs having a functional role on the RNA Polymerase II transcript mechanisms are expressed through RNA Polymerase III processing [6,7]. Following RNA Polymerase production, the individual lncRNA is subjected to 5' capping and other transcriptional editing such as pre-lncRNA splicing procedures and polyadenylation [6]. Final lncRNA developments essentially involve the formation of a stable secondary (and tertiary) structure, which confer the individual lncRNA its unique functional roles [6].

3. Functional Roles of LncRNAs

Presently there are already numerous regulatory (and other) roles for which lncRNAs have been identified to be responsible for, though such roles can be classified as either positive or negative expressions of gene regulation at either one of the transcriptional or post-transcriptional levels [5]. In addition, lncRNAs have been found to exert their functional roles through either acting on different regions on their host chromosome (cis-acting) or on other chromosomes (trans-acting) [8].

It is not the scope of this review to delve into great detail regarding all the varying functional roles of lncRNAs in general. However, the following sections will describe the evidence-based links between specific lncRNAs and their propensity to affect the course of action of individual and/or multiple tumours from a mechanistic viewpoint where possible.

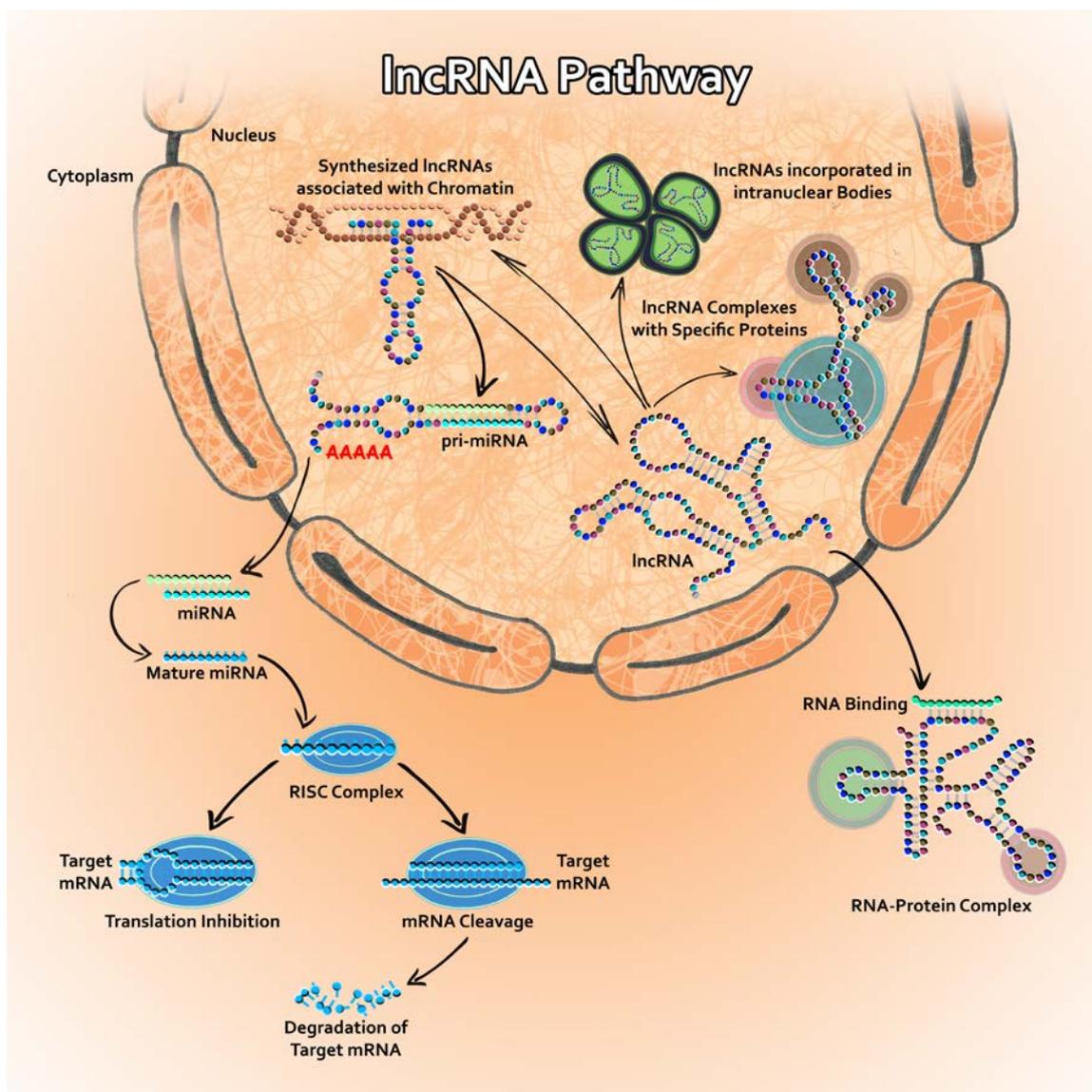


Figure 1. Overview of functional properties exhibited by lncRNAs. Among other functions, lncRNAs interact as scaffolding molecules to form RNA-protein complexes and can bind to specific chromatin regions epigenetically [31]

4. lncRNAs Acting as Biomarkers in Cancer

Recent research efforts have elucidated lncRNAs to be implicated into the tumorigenesis, tumour progression and also metastatic properties in a wide variety of human cancer conditions [9].

One of the most important prognostic biomarkers from this class of molecular family is undoubtedly the homeobox antisense intergenic RNA (HOTAIR) [10]. The HOTAIR lncRNA was identified to have up-regulated expression in gastric adenocarcinoma tissues [11]. The exacerbated HOTAIR expression level also correlated well with increased SUZ12 expression in the gastric adenocarcinoma patient group, thus suggesting that this co-expression could affect the epigenetic make-up of such tumour tissues [11]. A postulation for this would be that tumorigenesis – inducing gene expression pathways are activated by the HOTAIR – SUZ12 co-expression [11]. The increased HOTAIR expression could also, according

to the authors of the study, affect genomic relocalisation of the polycomb repressive complex 2 and also enhance trimethylation of H3K27 [11].

Apart from gastric adenocarcinomas, the HOTAIR lncRNA was also found to have clinical relevance in colorectal cancer conditions [12]. The results of the study by Kogo and colleagues highlighted exacerbated expression of HOTAIR in a cohort of 32 stage IV colorectal cancer biopsy tissues, with additional correlation to liver metastases [12]. Gene set enrichment analyses following cDNA array processing also demonstrated a highly significant correlation in the levels of expression between HOTAIR and members of the polycomb repressor complex 2, such as SUZ12, EZH2 and H3K27me3, thus suggesting polycomb-dependent chromatin modification activities induced by elevated HOTAIR expression [12]. Such evidence clearly places HOTAIR as a reliable biomarker for poor prognosis in colorectal cancer.

In addition to the above scientific evidence, HOTAIR has also been recognized as a biomarker for poor prognosis and tumour progression in hepatocellular carcinoma (HCC) [13,14,15]. The study by Yang and

colleagues on 110 HCC samples examined the expression levels of HOTAIR and additionally performed gene knockdown by RNAi technology in HCC cell lines to examine the effects of HOTAIR on multiple HCC tumour phenotypes [15]. The study concluded that HOTAIR expression is exacerbated in the HCC tissues compared to the surrounding non-cancerous tissues, with the HCC cell lines subjected to HOTAIR knockdown exhibiting a marked reduction in cell motility and viability and enhanced susceptibility to tumour necrosis factor alpha – based immune responses and also an increased chemosensitivity to conventional chemotherapeutic agents such as cisplatin and doxorubicin [15]. A similar study conducted by Geng and colleagues demonstrated exacerbated HOTAIR expression in 63 HCC samples and also highlighted a high correlation between HOTAIR expression and lymph node metastasis, thus also suggesting HOTAIR to be a prognostic biomarker for HCC metastasis [14].

Breast cancer was also correlated with HOTAIR activity in multiple studies [16,17]. The study carried out by Chisholm and colleagues demonstrated exacerbated HOTAIR expression in primary and metastatic breast carcinoma tissues obtained from formalin fixed paraffin-embedded sample archives, through RNA in situ hybridization techniques [17]. The study also highlighted the inference that increased HOTAIR lncRNA expression can affect the polycomb repressive complexes, resulting in tumour progression [17]. Another study focusing on HOTAIR activity in breast cancer revealed the dependence of HOTAIR expression on oestradiol production, due to its promoter region having several oestrogen response elements [18]. The study also demonstrated that HOTAIR knockdown induced apoptotic pathways in breast cancer cell lines and also suggested the oestrogen receptors as co-regulators for HOTAIR expression, leading to tumour progression [18]. Conversely to the evidence described above, a separate study focusing on 348 primary breast cancer biopsies analysed for intergenic DNA methylation and HOTAIR expression utilising RT-qPCR technology, stated that increased DNA methylation led to a reduction in HOTAIR expression and an unfavorable disease state, therefore acting as its regulator and ultimately questioning the suitability of HOTAIR as a negative prognostic biomarker in breast cancer diagnostics within the clinical setting [16].

Another major lncRNA key player in cancer is the metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) [19]. The study by Gutschner and colleagues developed a knockout for MALAT1 as a measure for developing a loss-of-function model for the lncRNA in lung tumour conditions [19]. Such a model revealed a marked reduction in metastatic capacities of lung adenocarcinoma murine xenografts deficient in MALAT1 expression [19]. Additionally, antisense oligonucleotide induced MALAT1 knockdown was successful in providing inhibition of metastasis after tumour implantation [19]. The conclusions of this study were that MALAT1 can be classified as a key prognostic biomarker for metastasis in lung adenocarcinoma conditions, with the lncRNA acting primarily as a gene regulator for metastasis-related genes [19].

Apart from lung adenocarcinoma, MALAT1 was implicated in other tumour conditions, such as

hepatocellular carcinoma (HCC) [20]. The study by Lai and colleagues focused analysing MALAT1 expression, using RT-qPCR, on nine HCC cell lines and 112 HCC tumour biopsies, of which 60 were post-liver transplant procedure [20]. Short interfering RNA (siRNA) knockdown of MALAT1 was also performed [20]. The results of this study demonstrated exacerbated MALAT1 expression in all patient group samples and HCC cell lines, together with a correlative link between MALAT1 up-regulated expression and post-liver transplant tumour re-emergence [20]. The knockdown of MALAT1 by siRNAs in HepG2 cell lines also demonstrated a marked reduction in tumour progression related phenotypes such as motility and viability, together with enhanced sensitivity to apoptosis, thus confirming MALAT1 as a reliable negative prognostic biomarker for HCC, particularly for tumour recurrence events post-liver transplant [20].

Furthermore to the above evidence, the detailed review article by Gutschner and colleagues serves to elucidate the influences of the MALAT1 lncRNA on several other tumour models, including osteosarcoma, uterine endometrial stromal sarcoma, together with cervical, bladder and colorectal cancer [21].

Another important lncRNA biomarker, that is identified for HCC in this case, is the long non-coding RNA for microvascular invasion in HCC (MVIH) [22]. The study conducted by Yuan and colleagues demonstrated up-regulated expression of MVIH in 215 HCC biopsies and revealed MVIH as a reliable biomarker for poor prognosis in HCC recurrence-free survival, specifically due to the influences of MVIH in angiogenesis within such tumour tissues [22]. This mechanism of tumour progression was confirmed since MVIH over-expression in murine HCC models resulted in the induction of angiogenesis and exacerbated intrahepatic metastasis [22]. In addition, microvessel density was proved to be exacerbated in correlation with MVIH expression [22].

Together with MVIH, the lncRNA H19 was also recognized as a reliable biomarker for HCC prognosis [23]. The study carried out by Matouk and colleagues highlighted, through H19 knockdown experiments, that H19 expression has a direct effect on *in vivo* HCC tumourigenicity [23]. This correlation was also identified for H19 expression in bladder cancer [23]. This study also identified several downstream targets for H19 activity, namely angiogenin and fibroblast growth factor 18 (FGF18) [23].

Prostate cancer is also affected by direct influence of oncogenic lncRNA activities. The study carried out by de Kok and colleagues revealed that the lncRNA DD3 was highly up-regulated in prostate tumour tissue samples and that the link was of sufficient degree to suggest DD3 as a negative prognostic biomarker for prostate tumour progression [24]. Another study, conducted by Chung and colleagues, identified a correlation between up-regulation of prostate cancer non-coding RNA 1 (PRNCR1) and prostate carcinoma tumourigenicity [25]. Furthermore, the study performed by Prensner and colleagues employed transcriptome sequencing techniques within a cohort of 102 cell lines and prostate cancer patient biopsies [26]. This study elucidated the up-regulation of the prostate cancer associated non-coding transcript 1 (PCAT-1) long intergenic non-coding RNA with tumour progression, therefore placing PCAT-1 as another potential negative

prognostic biomarker for prostate carcinoma conditions [26]. Conversely, within the same tumour model, other lncRNAs have been identified to act as tumour suppressors. The comprehensive study carried out by Polisenio and colleagues identified the lncRNA PTEN pseudogene 1 (PTENP1) to be down-regulated in prostate and colon carcinoma tumour models [27].

Other tumour suppressor lncRNAs were found to be of prognostic importance in multiple tumour models. The study performed by Huarte and colleagues highlighted the down-regulation of the lncRNA linc-p21 with tumour advancement in murine tumour models for sarcoma, lung adenocarcinoma and lymphoma [28]. In addition, the maternally expressed gene (MEG3) lncRNA was found to act as a tumour suppressor in several tumour conditions such as meningioma and glioma due to its down-regulated expression in severe forms of such tumour conditions [29,30].

5. Perspectives & Conclusions

The scientific evidence described above is simply the tip of the iceberg relating to the emerging importance of long non-coding RNAs as key biomarkers in cancer diagnostics and within the clinical setting. Ultimately, there will be soon be the establishment of quantitative high-throughput long non-coding RNA expression profile signature analyses on a par level with other molecular diagnostic techniques such as mRNA and miRNA expression profiles for the identification and monitoring of tumour progression and sensitivity to treatment within the individual cancer patient. Such lncRNA biomarkers can also serve as novel drug target blueprints for the development of novel, translational medicine approached cancer therapeutics in the not too distant future.

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Statement of Competing Interests

The author has no competing interests.

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