

Review Article

Long Non-Coding RNA MALAT1 Regulates Gene Expression and Protein Function via Multiple Layer and Flexible Manners

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Introduction

The Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1) is one of the most significant molecules of long non-coding RNA (lncRNA), also known as Nuclear Enriched Transcript2 (NEAT2). MALAT1 was first discovered as a prognostic marker for non-small cell lung carcinoma [1]. Soon after, researchers found MALAT1 is linked to other cancers such as endometrial cancer [2], breast cancer [3], cervical cancer [4], colorectal cancer [5], hepatocellular carcinoma [6], liver cancer [7], neuroblastoma [8], osteosarcoma [9], pancreatic cancer [10], prostate cancer [11], bladder cancer [12], gastric cancer [13] and etc. In addition to its role as a biomarker for many human tumors, MALAT1 was also identified as a critical regulatory molecule to control target gene expression, modify RNA and protein (enzyme) activity, as well as affect cellular distribution [14-16], consequently it is intimately associated with the regulation of cell growth and proliferation. Dysregulations of MALAT1 result in multiple tissue carcinogenesis as well as many other human disease processes [17-20]. As such the critical regulatory role and potential clinical implications of MALAT1 have attracted more and more attention recently [10,17]. This review will focus on the regulatory role and molecular mechanisms of MALAT1 on gene expression and biochemical function of proteins. In addition, the paper will put forward the concept that the regulatory mechanisms of lncRNA on the gene expression and target protein function are in multiple layer and flexible manner.

I: MALAT1 regulates the bioactivity of target proteins via direct protein-lncRNA interaction

The interaction of lncRNA with protein has been linked to the regulation of target protein bioactivity [21-25]. MALAT1 interacts

with several Alternative-Splicing (AS) factors such as SRSF1, SRSF2 and SRSF5 [26], which belong to a family of Serine/Arginine (SR)-rich splicing factors to regulate pre-RNA AS. Typically, SRSF proteins contain an RNA Binding Domain (RBD, also known as an RNA Recognition Motif, RRM) and Arginine/Serine (RS)-rich domain required for protein-protein interaction. SRSFs also regulate tissue or cell specific alternative splicing through a concentration and phosphorylation dependent manner. Full length MALAT1, as an abundant lncRNA molecule is localized in nuclear speckles and nucleoplasm, interacting with a sub-set of SRSF proteins and modulating their sub-nuclear distribution. It regulates cellular levels as well as the ratio of phosphorylated versus dephosphorylated SR proteins. Therefore MALAT1 plays a role in controlling alternative splicing patterns of certain endogenous pre-mRNAs. Ji Q. et al [27] demonstrated MALAT1 over expression in human Colorectal Cancer Cells (CRC), and discovered that MALAT1 binds to SFPQ (PTB-associated splicing factor), thus releasing proto-oncoprotein PTBP2 from SFPQ/PTBP2 complex, the increased SFPQ-detached PTBP2 then promoted cell proliferation and migration. The regulatory mechanism of protein activity by the interaction of MALAT1 and functional protein or complex is shown in Figure 1.

II: MALAT1 regulates gene transcription via modification of epigenetic program

MALAT1 involvement in transcriptional regulations is also mediated through the epigenetic processes. Yang et al. [28] reports MALAT1 can facilitate the assembly of multiple co-repressors/co-activators and find that MALAT1 alters the histone modifications on chromatin by alternating the activity of Polycomb2 protein (Pc2)

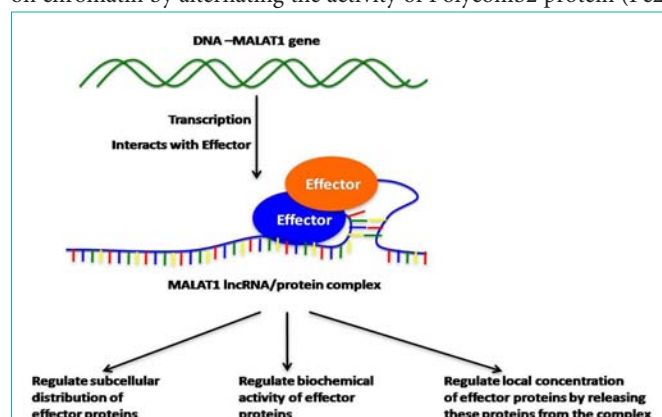


Figure 1: MALAT1 modifies the bioactivity of effector proteins.

MALAT1 molecule interacts with target proteins that contain RNA Binding Domain (RBD), such as alternative-splicing factors SRSF1, SRSF2, SRSF5 and SFPQ. It regulates the subcellular localization, enzymatic activity of these molecules and splicing behavior, and eventually affects the proliferation, differentiation and migration of cells.

in vitro. For in vivo conditions, the interaction between MALAT1 and Pc2 protein causes to release the target genes from repressed status (in polycomb bodies) to activated form (in interchromatin granules) in response to stimulation of growth signals. In addition, the binding of unmethylated Pc2 proteins with MALAT1 lncRNA promotes E2F SUMOylation therefore activating the transcription processes for genes associated with growth control. Thus MALAT1 plays a regulation role in gene expression via modification of epigenetic programs. In addition, MALAT1 molecule has been linked to physically interact with critical chromatin-modifier Polycomb Repressive Complex 2 (PRC2) to modulate the epigenetic status of target genes. Sonia G. et al [29] reports MALAT1 directly binds to EZH2 protein, which is a critical component of PRC2 complex to play methyltransferase activity of the chromatin histone modifications; Jason AW. et al [30] publishes similar result that MALAT1 binds to active chromatin sites, these experimental evidences show MALAT1 modulates the chromatin histone methylations by binding to PRC2 complex and abolish the it's methylation activity (Figure 2).

III: MALAT1 affects the gene expression via chromosome translocation and gene fusion

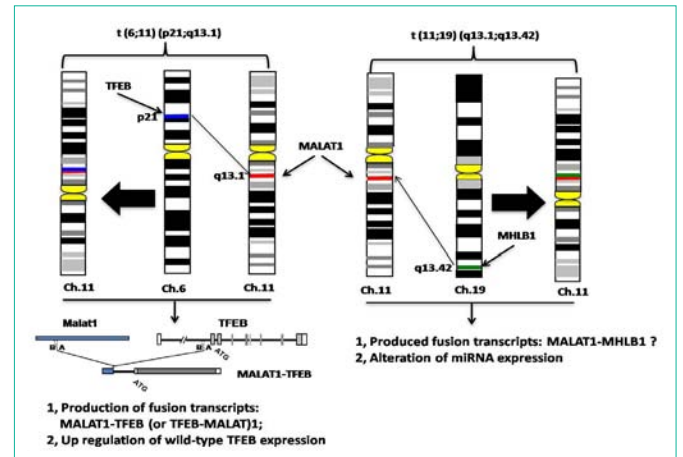
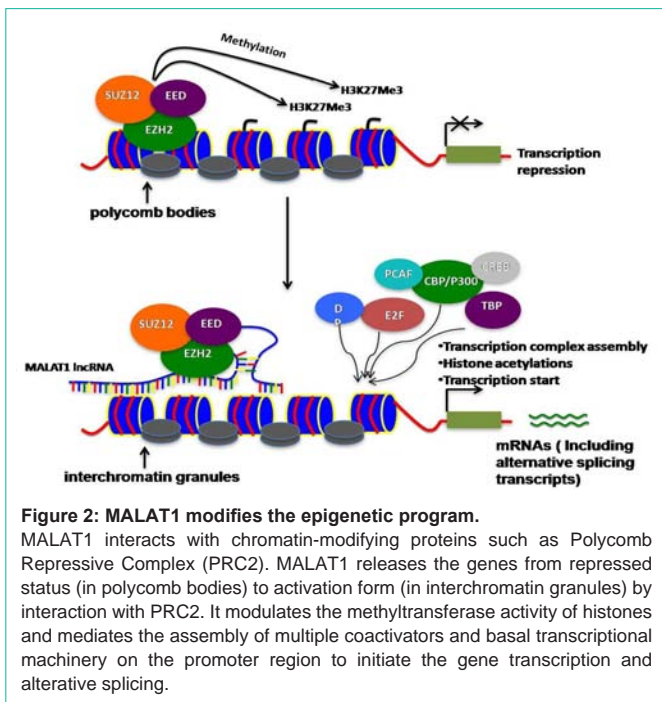
Chromosome translocation causes changes in structure and conformation of chromosomes, frequently resulting in the production of new chimeric RNA and fusion proteins, which subsequently affects the gene transcription/translation activity and biochemical function of target proteins. It has been reported that MALAT1 gene is often translocated in tumor cells. Rajaram V. et al. [31] reports that t (11; 19) (q13.1; q13.42) is identified in Mesenchymal Hamartoma of Liver (MHL). This translocation causes a breakpoint in MALAT1 gene on chromosome 11, resulting in mutated isoforms of MALAT1 RNA by the fusion of MALAT1 sequence with the DNA from a gene-poor region termed MHLB1 on chromosome 19. The result is the production of mutated isoforms of MALAT1-MHLB1. This translocation and disruption of the MHLB1 region may contribute to

MHL development through alterations in miRNA expression pattern. Renal cell carcinoma also often harbors MALAT1 translocations. Davis IJ. et al. [32] discovered t(6;11)(p21;q13.1) in a subset of renal cell carcinomas, this translocation causes MALAT1 (Alpha)-TFEB gene fusion, resulting in over-expression of native TFEB protein, which is not detectable under normal conditions. Therefore this translocation activates the TFEB expression or stabilizes the expression products (Figure 3).

IV: Potential mechanisms of MALAT1 gene expression regulation

MALAT1 as a typical lncRNA molecule and potentially regulates gene expression via similar pathways that exist in other long non-coding RNAs such as HOTAIR (HOX transcript antisense RNA) [33], AIRN (antisense of IGF2R non-protein coding RNA) [34,35] and BANCR (BRAF-activated non-protein coding RNA) [36] and etc. Some critical mechanisms have already been identified which are involved in expression regulation by these lncRNA molecules.

A) lncRNA interacts with gene promoters or enhancers to regulate transcription: The lncRNA-DNA-DNA triple helix was identified from lncRNA-chromatin interaction complex [37-40]. It has been reported that a GGUG-bearing lncRNAs (also called pncRNA, long promoter associated ncRNA) can recruit the RNA binding protein TLS (Translocation in Liposarcoma-associated) to the *cyclin D1* promoter region and induce a conformational change of TLS. TLS then interacts with CBP/p300 and inhibits their HAT activities [41]. In this type of regulation, a key consensus sequence of the pncRNA is composed of GGUG, but not every lncRNA sequence containing GGUG was targeted by TLS, this suggests that a secondary structure of the GGUG-bearing lncRNAs is also involved in recognition by TLS. Furthermore there is no direct evidence to show the short fragments or full length MALAT1 bind to promoter



DNA at present, it is nevertheless a reasonable prediction that MALAT1 may bind to some target promoter regions to regulate the transcription activity based on the fact that MALAT1 has plenty of GGUG conserved sequences (Figure 4a).

B) lncRNA Interacts with mRNA as the antisense strand to block target translational activity: Recent researches [42,43] show the lncRNA H19 is processed into microRNA fragments (called miR675), which target tumor suppressor retinoblastoma (Rb) mRNA and down regulates its translation. Similarly, intronic antisense lncRNA fragments are correlated with the degree of tumor differentiation in prostate cancer samples [44]. MALAT1 transcripts display distinct subcellular localizations that may be associated with the unique functions. For example, a highly conserved 61 bp tRNA-like small RNA molecule from full length MALAT1 is identified exclusively in the cytoplasm [45], these small RNAs are named MALAT1-associated small cytoplasmic RNAs (mascRNA). Some of these mascRNAs of MALAT1 possibly act as siRNA molecules to bind to complementary coding mRNA sequences, which then down-regulate gene expression by degrading the target mRNA transcripts (Figure 4b).

C) Regulation of gene expression via cis-splicing and trans-splicing processes: In eukaryotic cells, the primary transcripts include intronic and exonic sequences. The pre-mRNAs are processed by the spliceosome (splicing complex) to remove intronic sequences to form matured mRNA. When this process of intron removal occurs within the same pre-mRNA molecule, it is termed cis-RNA splicing. Splicing occurring in two separated pre-mRNA molecules is termed trans-RNA splicing. Both cis and trans RNA splicing processes follow similar mechanisms, which are catalyzed by the spliceosome [46]. Zhang, XO. et al [47] Recently reports *sno-lncRNAs* (intro-derived long noncoding RNAs with snoRNA [small nucleolar RNA] ends) are highly expressed from imprinted Prader-Willi Syndrome (PWS) region on human chromosome 15q11-q13. The *sno-lncRNAs* formation requires alternative splicing procedures, including cis and trans splicing. Thus a single gene can be spliced into multiple RNA transcripts. Using public database, the cis and trans variants of lncRNA including MALAT1 can be found [48,49], suggest similar mechanism may exist for MALAT1 functions (Figure 4c).

D) lncRNA interacts with basal transcriptional machinery to regulate transcription activity: It has been discovered that lncRNAs can interact with basal transcription machinery of cell to influence transcription activity. For example, a 331 bp 7SK lncRNA represses transcription elongation via preventing pTEF β transcription factor from phosphorylating the RNAPII Carboxyl-Terminal Domain (CTD), therefore regulating the transcription activity [50]. Other authors [51] reports that a 178-nucleotide called B2 lncRNA plays a role *via* binding with RNAPII to inhibit phosphorylation of its CTD by TFIIH kinase activity, therefore abolishing the binding ability of RNAPII to promoter sequences and as a result repressing transcription (Figure 4d).

E) lncRNA regulates gene expression and target protein activity through a multiple-layer and flexible manner: The regulatory manner of lncRNA shows a significant difference from the classic regulatory modality of protein factors.

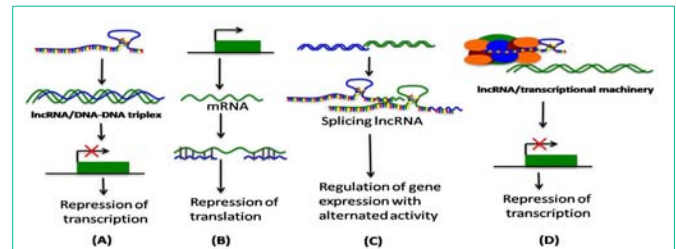


Figure 4: Potential mechanisms of MALAT1 gene expression regulation. Four different regulation mechanisms by lncRNAs are shown that are not identified in MALAT1 regulations, however, it is possible that these mechanisms also exist in MALAT1 regulations. (A). lncRNA molecule interacts with double strand DNA and represses gene transcription; (B). lncRNA fragments act as intronic siRNA to bind with mRNA and repressing mRNA translation; (C). Produce alternative splicing lncRNAs to regulate gene expression. Different isoforms from alternative splicing have different regulation activity and specificity, which regulate the gene expression with different patterns; (D). lncRNA molecule interacts with basal transcriptional machinery which disrupts the transcription initiation complex and represses transcription.

i) As lncRNA genes can easily produce different alternatively spliced isoforms from a single and fixed DNA sequence [47,52], it has been shown that the inflammatory signaling via TNF- α in mouse cells [53] can induce lncRNAs and its isoforms

ii) lncRNA can change subcellular localization by migrating from nuclei to cytoplasm. For example, the full length MALAT1 is localized in nuclear speckles while small fragments derived from MALAT1 cleavage are localized in the cytoplasm. This kind of migration offers the ability of lncRNA molecule to interact not only with nuclear DNA or proteins, but also with cytoplasm mRNAs and protein factors [45,54], therefore, a single lncRNA molecule can play regulatory function at different stages of gene expression including transcription, translation and Posttranslational Modifications (PTM) of proteins. In addition to the regulations derived from interactions between lncRNA and target macromolecules, the regulations by lncRNAs are also affected by the interactions with the chemical environment including pH value, salt concentrations and other small molecules. All of these factors will affect the stability of and potential for such interactions.

Gene regulations by lncRNA via flexible and multiple-layer manners signify the importance of physiological functions in efficiently balancing gene expression and biological behavior. Controlling or managing these regulations may also serve as new therapeutic strategies to different diseases including many types of cancers. The clinical implication and therapeutic potential of MALAT1 have attracted researchers to explore the down-regulation of this target and the results are encouraging. For example, Ren S. et al [55] reports *in vitro* and *in vivo* results of down-regulation for MALAT1 expression by siRNA technique, they find that for cultured prostate cells, down-regulation of MALAT1 inhibits prostate cancer cell growth, invasion and migration, as well as induces G0/G1 phase arrest. *In vivo*, intratumor delivery of therapeutic siRNA targets MALAT-1, delays tumor growth and reduces metastasis of cancer cell xenografts in castrated male nude mice. Surprisingly, this effect is consistent across different tumor types, Gutschner, TM. et al. [16] reports that targeted MALAT1 of lung cancer cells using siRNA inhibits metastasis formation after tumor implantation *in vivo*. Lai,

MC. et al. [56] reports in a Hepatocellular Carcinoma (HCC) tumor cell line (HepG2), inhibition of *MALAT1* could effectively reduce cell viability, motility, invasiveness, and increase the sensitivity to apoptosis. The clinical implications of *MALAT1* have also been extended to other diseases. Watts, R. et al. [57] reports that knockdown of *MALAT1* using siRNA suppresses myoblast proliferation by arresting cell growth in G0/G1 phase. Therefore target inhibition of *MALAT1* shows potential for regenerative biology in the treatment of muscle atrophy and muscle wasting diseases. *MALAT1* not only opens new avenues in considerations of gene expression regulation, it also potentially serves as one of the best predictive biomarker for many tumors. But perhaps most importantly, it provides a potential target for new and critical clinical therapeutics.

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