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Hox transcript antisense intergenic RNA possible role in diagnosis of Prostate Cancer

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Abstract

Prostate cancer is the second most common cancer diagnosed in men globally, after lung cancer. The discrepancy of about 20,000 protein-coding genes and over 100,000 different transcripts identified in mammalian transcriptomes highlights the possibility of discovering a novel class of non-translated RNAs, beyond those already identified in the 1970s, as part of the translation machinery: ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs). Non-coding RNAs (ncRNAs) can be grouped according to their length, localization, and/or function: long non-coding RNAs (lncRNAs), microRNAs (miRNAs), small interfering RNAs (siRNAs), small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs), and PIWI-interacting RNAs (piRNAs). Some lncRNAs present unique requirements for their proper expression and functionality, which can be mediated by cMyc TF and Dicer, a key enzyme involved in miRNA processing. (Hox transcript antisense intergenic RNA) HOTAIR, first identified from a custom tilling array of the HOXC locus, is encoded from the HOXC locus on chromosome 12q13. It belongs to the long non-coding RNAs, with 2,158 nucleotides. HOTAIR RNA does not encode any proteins, however it is important in gene regulation by modifying chromatin structure. HOTAIR is overexpressed in multiple types of cancers, and its overexpression is associated with metastasis and poor survival rates. HOTAIR acts as an androgen-repressed lncRNA. Early studies indicated that androgen receptor (AR) was not only important for the growth and differentiation of a healthy prostate but also played a crucial role in the pathogenesis of PCa. HOTAIR induced AR activation in an androgen-independent manner and played an important role for growth and invasion in PCa cells. HOTAIR also suppressed AR signaling by regulating the PRC2 complex, consequently increasing the stem/progenitor cell population and invasion in PCa cells. Thus, modulation of the PRC2 complex with HOTAIR at the AR promoter region would affect PCa resistance to androgen deprivation therapy. Moreover, HOTAIR was shown to enhance the AR-mediated transcriptional program and drive castration-resistant prostate cancer (CRPC).

Keywords: Hox transcript antisense intergenic RNA, Prostate Cancer

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1. Introduction

Prostate cancer is the second most common cancer diagnosed in men globally, after lung cancer [1]. Despite relatively high survival rates for men with prostate cancer, more than 300,000 prostate cancer deaths occurred in 2012 worldwide [2]. Age and family history are key risk factors for prostate cancer, and black men have a higher risk of prostate cancer incidence and death compared to men from white or Asian backgrounds [2]. Majority of cases of prostate cancer are diagnosed in men from western countries in Americas and Europe, and this has largely been driven by introduction of prostate-specific antigen (PSA) for prostate cancer detection in the 1990s [3]. The widespread use of PSA has proven controversial as evidence for benefit as a screening test in asymptomatic men is still subject to debate, and PSA is prone to false positives and false negatives in men with symptoms suggestive of a possible diagnosis of prostate cancer [3]. Most prostate cancer diagnoses are made in symptomatic men. Prostate cancer should be suspected in men over 50 years old presenting with lower urinary tract symptoms (LUTS), visible haematuria or erectile dysfunction [4].

Lower urinary tract symptoms are also a common presenting symptom of benign conditions affecting the prostate, such as benign prostatic hyperplasia (BPH) and prostatitis, creating a diagnostic challenge. There is no strong evidence of association between the severity of LUTS and the likelihood of prostate cancer or the stage at diagnosis [5]. Digital rectal examination (DRE) is recommended in many countries alongside PSA to aid decision-making about referral for diagnostic testing. A recent systematic review suggests that DRE has a high specificity and positive predictive value (PPV) for prostate cancer in symptomatic

patients [6]. In light of the limitations of PSA, a number of other tests have been investigated to aid the diagnosis of clinically significant prostate cancer. PSA is a kallikrein serine protease, and other related biomarkers have been assessed for a potential role in prostate cancer detection [7]. Multiparametric magnetic resonance imaging (mpMRI) has gained much interest in recent years, both as a diagnostic test for prostate cancer and for monitoring men with localized prostate cancer on active surveillance for signs of disease progression [8].

2. Long non-Coding RNA

The discrepancy of about 20,000 protein-coding genes and over 100,000 different transcripts identified in mammalian transcriptomes highlights the possibility of discovering a novel class of non-translated RNAs, beyond those already identified in the 1970s, as part of the translation machinery: ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) [9]. The transcriptome data from cellular lineages and human tissue samples showed that at least 60% of the genome is expressed as primary or processed transcripts, much more than previously predicted. This analysis revealed that thousands of unannotated RNAs may act as non-coding regulatory elements in gene expression or originate as small RNAs [10]. Non-coding RNAs (ncRNAs) can be grouped according to their length, localization, and/or function: long non-coding RNAs (lncRNAs), microRNAs (miRNAs), small interfering RNAs (siRNAs), small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs), and PIWI-interacting RNAs (piRNAs) [11].

I. lncRNA biogenesis and transcription

The lncRNAs are larger than 200 nucleotides and can be subdivided according to their biogenesis loci:

- Intergenic lncRNAs (lincRNAs) [12].
- Intronic lncRNAs
- Antisense lncRNAs (as lncRNA or natural antisense transcripts, NATs) [13].
- Bidirectional lncRNAs
- Enhancer RNAs (eRNAs).

The lncRNA biogenesis is mostly similar to messenger RNA (mRNA), since this process is also mediated through RNA polymerase II. In addition, lncRNAs can appear with or without polyadenylation, alternative cleavage, alternative polyadenylation, and alternative splicing, leading to different isoforms from the same locus [14-15]. The lncRNAs genes share features with coding-genes promoters and can be regulated by some transcription factors (TFs), such as p53, nuclear factor-kappa B (NF-κB), Sox2, and Oct4 [16]. Some lncRNAs present unique requirements for their proper expression and functionality, which can be mediated by cMyc TF and Dicer, a key enzyme involved in miRNA processing [17]. The antisense lncRNAs originate from the complementary strand of protein-coding genes [18], about 30% of human annotated genes have an antisense component that highly impacts gene profile expression [19]. Divergent transcription can occur when RNA polymerase II is recruited in the antisense strand at an upstream site of the protein coding gene promoter, but only a fraction of them generate functional transcripts, called bidirectional lncRNAs [18]. Evaluation of lncRNAs in human and murine embryonic stem cells showed that over 60% were divergently transcribed and the changes are coordinated with cognate protein coding genes during differentiation [20]. Cap analysis of gene expression (CAGE) showed that eRNAs are bidirectionally transcribed and capped [21], but non-polyadenylated and non-spliced, depending on the integrator complex for the 3' end cleavage of transcript [22]. Circular RNAs (circRNAs) were predicted to be the most abundant isoform compared to linear transcripts. The circRNAs can be formed via regular splicing (intronic circRNAs) and via non-canonical splicing, joining the splice donor at an upstream acceptor site (backsplicing) [23]. Some isoforms of circRNAs can be derived from circularization of an intronic fragment with their neighbor exons, forming exon-intron-circRNA (elciRNAs), which can associate with RNA polymerase II and with U1 snRNA, increasing transcription of their parental genes [24].

The highly intricate mechanisms to regulates lncRNA degradation leads to specific expression in different cellular types and seems to be subjected to the same mechanisms correlated with mRNA decapping, or alternatively through nonsense-mediated decay (NMD) [25]. Expression of lncRNAs can be detected from uni- to pluri-cellular eukaryotic organisms, although the processing and mechanisms of action can differ [26]. The most recent NONCODE database source points to over 100,000 lncRNAs in the human genome, but this number seems to be underestimated [27]. Cellular and temporal specificity drives the mechanism of action of lncRNAs and their simultaneous impact in diverse target genes [28]. Long non-coding RNA can regulate neighbor protein-coding genes expression and thus contribute to the mRNA and protein content in the cell [29]. Long non-coding RNAs are found within the nucleus, nucleolus, cytoplasm, and even in the mitochondria [30] and its localization is a good indicator of their mode of action [9]. lncRNAs have been associated with diverse functions. Their biological contributions have been seen in the form of: Regulators of transcription in cis or Trans; Modulators of mRNA processing, post-transcriptional control and protein activity; and Organization of nuclear domains [31].

II. Mechanism of action

The regulation of gene expression in eukaryotes is complex and compartmentalized. It can occur in multiple steps, such as in the chromatin organization, transcription machinery recruitment, mRNA processing and its delivery to the cytoplasm, mRNA half-life, translation, and post-translational processes, which can be interfered with by lncRNAs [32].

Chromosome and Chromatin Structure: The idea that RNA can be a chromatin-associated structural component was corroborated by the description that, there, the amount of RNA is twice as high as the DNA associated with the chromatin structure. Many studies identified several types of RNAs related to this function, such as snRNAs, and lncRNAs, such as the X inactivation-specific transcription (XIST), AIR, and H19, were associated with heterochromatin formation and imprinting [33]. Additionally, lncRNAs that are expressed only in embryonic stem cells, interact directly with the chromatin, then modulate gene expression and the maintenance of pluripotency [16]. The lncRNA interaction with DNA can occur by sequence complementarity to a single-stranded fragment of DNA or allocation in the helix [27]. Additionally, eRNAs can execute their function by mediating chromosomal looping together with the mediator complex [22].

- ***Transcription At the transcriptional level***

At the transcriptional level, the promoter region of an lncRNA sequence, regardless of its synthesis, can act as an enhancer, characterizing a cis regulation [34]. The NAT as Oct4-pg5 can indirectly regulate epigenetic markers through the RNA/DNA binding protein PURA (purine-rich element binding factor A), which reduces transcription from the protein-coding sense transcripts and simultaneously represses other NATs in a negative-feedback loop [35]. Some ncRNAs can interact directly with the transcription machinery, as shown by circRNAs that directly interact with RNA pol II, according to crosslink followed by immunoprecipitation assays [27]. Additionally, eRNAs can bind to transcription factors, positioning them in specific promoters [36]. Other lncRNAs can regulate transcription, controlling DNA methyltransferases recruitment, TFs, zinc-finger proteins, and others transcription regulators [9].

- ***Post-Transcriptional Regulation***

Long Non-Coding RNA and MicroRNA Interplay: Different classes of ncRNAs can interact through sequence complementarity by executing coordinated functions. The most remarkable interplay occurs between lncRNAs and miRNAs in the regulation of gene expression. Long non-coding RNAs can be endogenous competitors RNAs (ceRNAs), also called miRNA sponges, by presenting binding sequences for miRNAs [37] and can impair the functional interaction of miRNA and mRNA by interference in the gene regulation. Both linear and circular isoforms can exert this function. The first description of competition between these molecules involved the naturally-expressed circular RNA sponge for miR-7 (ciRS-7), via miRNA-dependent binding to argonaute (AGO) proteins [38]. During myogenesis, the linear transcript named lnc-MG competes with miR-125b, controlling insulin-like growth factor 2 (IGF-2) levels. Long non-coding RNAs can be precursors of miRNAs and can regulate different points of miRNA biogenesis, acting on microprocessor activity to finish the primary transcript in a mechanism independent of polyadenylation [39].

*Alternative Splicing: lncRNAs are described as interacting with splicing factors, composing duplexes of pre-mRNAs and antisense lncRNAs or chromatin remodeling, which can directly influence the transcriptional rate by RNA polymerase II and modulate splicing [40].

*Messenger RNA Stability: Long non-coding RNAs have properties capable of mediating the nonsense-mediated mRNA decay (NMD) pathway, in a miRNA-independent way. This function is exemplified by the complex formed by lncRNA half-STAU1-binding site RNAs (1/2-sbsRNAs) with the target mRNA, creating a double-stranded (ds) transactivation motif that interacts with the STAU1-dsRNA binding protein, leading to mRNA degradation. Some NATs can also decrease the stability of the protein-coding sense transcript [41.

Translation: Translation processes can be facilitated or repressed by lncRNAs, as shown by the dopaminergic neuron-specific expression of Uchl1 (ubiquitin carboxy-terminal hydrolase L1), which is regulated by its antisense transcript (AS Uchl1), which recruits polysomes by the repetitive domain SINEB2 to promote a cap-independent translation [42].

III. Physiological Conditions and Disease

The roles of lncRNAs in genome integrity and gene expression have demonstrated the relevance of these molecules for physiological and pathological conditions [43]. lncRNAs are involved in chromosomal compensation, imprinting, chronic diseases, immune response process, and in some pathogens. Long non-coding RNAs are correlated with different aspects of complex diseases and differential expression was observed in patients with neurodegeneration, such as Alzheimer's, Huntington's, and Parkinson's diseases; schizophrenia, and autism spectrum disorders. Dysregulation is frequently described in cardiovascular diseases, such as during chronic heart failure, diabetic cardiomyopathy, atherosclerosis, and infarction. The complexity of lncRNA regulation and specificity are being revealed as important determinants in diabetes mellitus. The same innate mechanisms of action of lncRNAs can lead to malignant transformation of the cell if expressed in moments distinct from the physiological ones [19].

3. HOTAIR

1-Structure and biological function of HOTAIR

(Hox transcript antisense intergenic RNA) HOTAIR, first identified from a custom tilling array of the HOXC locus, is encoded from HOXC locus on chromosome 12q13 [44]. It belongs to the long non-coding RNAs, with 2,158 nucleotides. HOTAIR RNA does not encode any proteins, however it is important in gene regulation by modifying chromatin structure [45]. In human, it is only transcribed from the antisense strand of the HOXC genes and partly overlaps with. Despite the fact that nascent forms of this transcript could be spliced, capped and polyadenylated using RNA polymerase II, they do not generate any functional protein [44]. HOTAIR has been manifested as the first lincRNA with trans-binding regulatory capability, contributing to regulation of the distant genes. Evolutionarily, transcription of HOTAIR has only been determined in mammals, including all vertebrates [45]. In the genome context, secondary structure of the HOTAIR gene body (including exonic and intronic regions), not only coordinates in the establishment of different transcription variants, but also associates with regulation of HOTAIR expression levels. In addition to the body structure, flanking regions of this lincRNA might also contribute to the regulation of HOTAIR expression. For instance, as a suppressor protein, interferon regulatory factor 1 (IRF1) could bind into the related binding motifs of HOTAIR promoter at two positions of 53–64 and 136–148 bp, upstream of transcription start site [48].

2-Function of HOTAIR in normal development

In human tissues, HOTAIR is highly expressed in skin and genital system (including testis, endometrium and prostate respectively). In addition to these tissues, expression of HOTAIR has been detected in lymph node, placenta, kidney, fat originating from mesenchymal cells and bladder, however, this expression could be tissue- or cell-dependent in some organs. As a case, among reproductive system tissues, expression of HOTAIR observed in testis and endometrium, but not ovary. Further investigations have also revealed that HOTAIR expression in skin depends on positional identity of fibroblast. Thus, foreskin and foot fibroblasts could express this lincRNA, in contrast to chest, lung and forearm [44].

Table 1. Changes in the expression of HOTAIR in different types of human cancer

Type of cancer	Expression Effect	invasion/metastasis	Reference
Breast cancer	Increased	Promote	**(Lu *et al.*, 2012)**
Esophageal cancer	Increased	Promote	**(Ge *et al.*, 2013)**
Lung cancer	Increased	Promote	**(Liu *et al.*, 2013)**
Gastric cancer	Increased	Promote	**(Endo *et al.*, 2013)**
Liver cancer	Increased	Promote	**(Ishibashi *et al.*, 2013)**
Prostate cancer	Increased	Promote	**(He *et al.*, 2014)**
Laryngeal cancer	Increased	Promote	**(Li *et al.*, 2013)**
Pancreatic cancer	Increased	Promote	**(Kim *et al.*, 2013)**
Colorectal cancer	Increased	Unknown	**(Kogo *et al.*, 2011)**
Nasopharyngeal cancer	Increased	Promote	**(Nie *et al.*, 2013)**

In addition to making scaffolds and/or platforms to enable interactions of DNA with multiplex proteins, evidences demonstrate critical effect of HOTAIR activity on cell cycle progression and proliferation by regulating different molecules. Transcription of this lincRNA could control expression of different cell cycle-dependent kinase, namely CDK2 and CDK4 as well as Cyclin E and Cyclin D1 [49]. It has been shown that HOTAIR contributes to the function of Cyclin D1 through activity of STAT3. Although, the mechanism of this procedure still remains unclear, it is proposed that HOTAIR coordinates in a molecular pathway leading to promoting proliferation through activation of CDK1/CDK2/STAT3 signalling cascade. CDK1 and CDK2 phosphorylate a threonine of EZH2 protein, as an important residue for appropriate function of this protein, in the context of PRC2 complex [50]. Interaction of HOTAIR with PRC2 complex promotes methylation in STAT3.

This methylation plays a role in phosphorylation of STAT3 tyrosine residue and activity of this protein [51]. Collaboration of Cyclin D1 with CDK4 and CDK6 contributes to post-translational phosphorylation of some proteins and activity of some necessary transcription factors for transition of G1 to S cell cycle. Consistent to this hypothesis, findings revealed that down-regulation of HOTAIR could promote G1 cell cycle arrest [52]. So that, loss of this lincRNA promotes expression of p27 leading to binding and prohibiting Cyclin D-CDK4 and Cyclin E-CDK2 activities. In addition to the key effect of HOTAIR on the activity of STAT3, findings suggest a positive feedback loop of STAT3 on promoter region of HOTAIR and elevating the lincRNA expression [53]. Expression of HOTAIR could also be regulated by interaction of IRF-1 transcription factor with promoter of this lincRNA. This mechanism leads to down-regulation of HOTAIR [54].

3-HOTAIR status in human malignancies

HOTAIR is overexpressed in multiple types of cancers, and its overexpression is associated with metastasis and poor survival rates. The regulation and function of HOTAIR in cancer is summarized in Table 1.

4. HOTAIR in prostate cancer

Prostate cancer (PCa) is the most common malignancy in men. LncRNAs can promote castration resistance, cell proliferation, invasion, and metastasis in PCa [54]. LncRNAs are newly deciphered “codes” and "special emphasis" in PCa, and an understanding of the role of lncRNAs, while only in its infancy, should present ample opportunities for the discovery of new biomarkers and therapeutic targets in PCa [53]. HOTAIR acts as an androgen-repressed lncRNA. Early studies indicated that androgen receptor (AR) was not only important for the growth and differentiation of a healthy prostate but also played a crucial role in the pathogenesis of PCa. HOTAIR induced AR activation in an androgen-independent manner and played an important role for growth and invasion in PCa cells. HOTAIR also suppressed AR signaling by regulating the PRC2 complex, consequently increasing the stem/progenitor cell population and invasion in PCa cells. Thus, modulation of the PRC2 complex with HOTAIR at the AR promoter region would affect PCa resistance to androgen deprivation therapy [50]. Moreover, HOTAIR shown to enhance AR-mediated transcriptional program & drive castration-resistant prostate cancer (CRPC). Microarray analysis for expression profiling indicated that HOTAIR was highly regulated by genistein, a soy-derived isoflavanoid compound acting as a chemotherapeutic agent against several cancer types.

In the link to miRNA, silencing of HOTAIR decreased proliferation, migration, invasion, and induced apoptosis; miR-34a directly targeted HOTAIR in PCa cells, which showed that genistein inhibited the growth of PCa cells by downregulating oncogenic HOTAIR that was also targeted by tumor suppressor miR-34a [53]. In addition, miR-193a acted as a tumor suppressor, inhibiting growth, migration and invasion in CRPC cells via the regulatory feedback loop of HOTAIR/EZH2/miR-193a. Thus targeting this aberrantly activated loop could provide a potential therapeutic strategy in PCs [52]. Polyphyllin I (PPI), one of the steroidal saponins in Paris polyphylla, reportedly exhibited antitumor activities. It was recently observed that PPI inhibited growth of CRPC cells by inhibiting HOTAIR expression, thereby repressing EZH2 and DNMT1. This result revealed a novel mechanism for HOTAIR-mediated regulation of DNMT1 and EZH2 in response to PPI in inhibiting growth of CRPC cells. Whether this risk referred to a risk locus for occurrence & development of PCa remained inconclusive. Together, aforementioned observations suggested that HOTAIR could potentially be a novel biomarker for diagnosis and prognosis of PCa. An understanding of the role of lncRNAs, including HOTAIR, in pathogenesis of PCa may unveil better opportunities for

discovery of new biomarkers, therapeutic targets, & potential implication of PCa in clinical setting [53].

- ***HOTAIR regulates the invasion and metastasis of prostate cancer by targeting hepaCAM***

HOX transcript antisense RNA (HOTAIR) is closely related to tumor metastasis and poor recurrence-free survival. Moreover, studies have identified HOTAIR as a novel diagnostic and prognostic biomarker [54]. The specific mechanisms underlying the metastatic regulation of HOTAIR in PCa are largely unclear. Therefore, the purpose of this study was to elucidate the role of HOTAIR in PCa and to provide alternative strategies for the development of targeted treatment and individualized treatment. Elevated HOTAIR promotes PCa metastasis by targeting an immunoglobulin-like cell adhesion molecule: hepatocellular adhesion molecule (hepaCAM). HepaCAM is a transmembrane glycoprotein, and the extracellular region plays an important role in regulating cell adhesion and movement [54].

References

[1] J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D.M. Parkin, D. Forman, F. Bray. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International journal of cancer. 136(5): E359-E386.

[2] M. Arnold, J. Ferlay, M.I. van Berge Henegouwen, I. Soerjomataram. (2020). Global burden of oesophageal and gastric cancer by histology and subsite in 2018. Gut. 69(9): 1564-1571.

[3] D.C. Grossman, S.J. Curry, D.K. Owens, K. Bibbins-Domingo, A.B. Caughey, K.W. Davidson, C.A. Doubeni, M. Ebell, J.W. Epling, A.R. Kemper. (2018). Screening for prostate cancer: US Preventive Services Task Force recommendation statement. Jama. 319(18): 1901-1913.

[4] NICE. (2015). National Institute for Health and Care Excellence Suspected cancer: recognition and referral.

[5] J.Ø. í Jákupsstovu, J. Brodersen. (2018). Do men with lower urinary tract symptoms have an increased risk of advanced prostate cancer? Bmj. 361: k1202.

[6] D. Jones, C. Friend, A. Dreher, V. Allgar, U. Macleod. (2018). The diagnostic test accuracy of rectal examination for prostate cancer diagnosis in symptomatic patients: a systematic review. BMC family practice. 19: 1-6.

[7] D.J. Arekh, S. Punnen, D.D. Sjoberg. (2015). A multi-institutional prospective trial in the USA confirms that the 4Kscore accurately identifies men with high-grade prostate cancer. Eur Urol. 68(3): 464-70.

[8] H.U. Ahmed, A.E.-S. Bosaily, L.C. Brown, R. Gabe, R. Kaplan, M.K. Parmar, Y. Collaco-Moraes, K. Ward, R.G. Hindley, A. Freeman. (2017). Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. The Lancet. 380(10071): 1-8.

[9] Y. Long, X. Wang, D.T. Youmans, T.R. Cech. (2017). How do lncRNAs regulate transcription? Science Advances. 3(9): eaao2110.

[10] S. Djebali, C.A. Davis, A. Merkel, A. Dobin, T. Lassmann, A. Mortazavi, A. Tanzer, J. Lagarde, W. Lin, F. Schlesinger. (2012). Landscape of transcription in human cells. Nature. 489(7414): 101-108.

[11] I. Ulitsky, D.P. Bartel. (2013). lincRNAs: genomics, evolution, and mechanisms. Cell. 154(1): 26-46.

[12] M.N. Cabili, C. Trapnell, L. Goff, M. Koziol, B. Tazon-Vega, A. Regev, J.L. Rinn. (2011). Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes & development. 25(18): 1915-1927.

[13] K. Li, R. Ramchandran. (2010). Natural antisense transcript: a concomitant engagement with protein-coding transcript. Oncotarget. 1(6): 447-452.

[14] J.C. Fernandes, S.M. Acuña, J.I. Aoki, L.M. Floeter-Winter, S.M. Muxel. (2019). Long non-coding RNAs in the regulation of gene expression: physiology and disease. Non-coding RNA. 5(1): 17.

[15] J.J. Quinn, H.Y. Chang. (2016). Unique features of long non-coding RNA biogenesis and function. Nature reviews genetics. 17(1): 47-62.

[16] M. Guttman, I. Amit, M. Garber, C. French, M.F. Lin, D. Feldser, M. Huarte, O. Zuk, B.W. Carey, J.P. Cassady. (2009). Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature. 458(7235): 223-227.

[17] G.X. Zheng, B.T. Do, D.E. Webster, P.A. Khavari, H.Y. Chang. (2014). Dicer-microRNA-Myc circuit promotes transcription of hundreds of long noncoding RNAs. Nature structural & molecular biology. 21(7): 585-590.

[18] M. Uesaka, O. Nishimura, Y. Go, K. Nakashima, K. Agata, T. Imamura. (2014). Bidirectional promoters are the major source of gene activation-associated non-coding RNAs in mammals. BMC genomics. 15: 35.

[19] Y. Zhang, X.S. Liu, Q.-R. Liu, L. Wei. (2006). Genome-wide in silico identification and analysis of cis natural antisense transcripts (cis-NATs) in ten species. Nucleic acids research. 34(12): 3465-3475.

[20] A.A. Sigova, A.C. Mullen, B. Molinie, S. Gupta, D.A. Orlando, M.G. Guenther, A.E. Almada, C. Lin, P.A. Sharp, C.C. Giallourakis. (2013). Divergent transcription of long noncoding RNA/mRNA gene pairs in embryonic stem cells. Proceedings of the National Academy of Sciences. 110(8): 2876-2881.

[21] R. Andersson, C. Gebhard, I. Miguel-Escalada, I. Hoof, J. Bornholdt, M. Boyd, Y. Chen, X. Zhao, C. Schmidl, T. Suzuki. (2014). An atlas of active enhancers across human cell types and tissues. Nature. 507(7493): 455-461.

[22] F. Lai, A. Gardini, A. Zhang, R. Shiekhattar. (2015). Integrator mediates the biogenesis of enhancer RNAs. Nature. 525(7569): 399-403.

[23] J. Salzman, C. Gawad, P.L. Wang, N. Lacayo, P.O. Brown. (2012). Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. Plos one. 7(2): e30733.

[24] Z. Li, C. Huang, C. Bao, L. Chen, M. Lin, X. Wang, G. Zhong, B. Yu, W. Hu, L. Dai. (2015). Exon-intron circular RNAs regulate transcription in the

nucleus. Nature structural & molecular biology. 22(3): 256-264.
[25] M. Wery, M. Descrimes, N. Vogt, A.-S. Dallongeville, D. Gautheret, A. Morillon. (2016). Nonsense-mediated decay restricts LncRNA levels in yeast unless blocked by double-stranded RNA structure. Molecular cell. 61(3): 379-392.
[26] Z.T. Beck, Z. Xing, E.J. Tran. (2016). LncRNAs: Bridging environmental sensing and gene expression. RNA biology. 13(12): 1189-1196.
[27] Y.G. Chen, A.T. Satpathy, H.Y. Chang. (2017). Gene regulation in the immune system by long noncoding RNAs. Nature immunology. 18(9): 962-972.
[28] S.J. Liu, M.A. Horlbeck, S.W. Cho, H.S. Birk, M. Malatesta, D. He, F.J. Attenello, J.E. Villalta, M.Y. Cho, Y. Chen. (2017). CRISPRi-based genome-scale identification of functional long noncoding RNA loci in human cells. Science. 355(6320): eaah7111.
[29] J. Joung, J.M. Engreitz, S. Konermann, O.O. Abudayyeh, V.K. Verdine, F. Aguet, J.S. Gootenberg, N.E. Sanjana, J.B. Wright, C.P. Fulco. (2017). Genome-scale activation screen identifies a lncRNA locus regulating a gene neighbourhood. Nature. 548(7667): 343-346.
[30] M.N. Cabili, M.C. Dunagin, P.D. McClanahan, A. Biaesch, O. Padovan-Merhar, A. Regev, J.L. Rinn, A. Raj. (2015). Localization and abundance analysis of human lncRNAs at single-cell and single-molecule resolution. Genome biology. 16: 20.
[31] S. Geisler, J. Coller. (2013). RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. Nature Reviews Molecular Cell Biology. 14(11): 699-712.
[32] F. Kopp, J.T. Mendell. (2018). Functional classification and experimental dissection of long noncoding RNAs. Cell. 172(3): 393-407.
[33] J.L. Rinn, H.Y. Chang. (2012). Genome regulation by long noncoding RNAs. Annual review of biochemistry. 81(1): 145-166.
[34] J.M. Engreitz, J.E. Haines, E.M. Perez, G. Munson, J. Chen, M. Kane, P.E. McDonel, M. Guttman, E.S. Lander. (2016). Local regulation of gene expression by lncRNA promoters, transcription and splicing. Nature. 539(7629): 452-455.
[35] P.G. Hawkins, K.V. Morris. (2010). Transcriptional regulation of Oct4 by a long non-coding RNA antisense to Oct4-pseudogene 5. Transcription. 1(3): 165-175.
[36] J.G. Azofeifa, M.A. Allen, J.R. Hendrix, J.D. Rubin, R.D. Dowell. (2018). Enhancer RNA profiling predicts transcription factor activity. Genome research. 28(3): 334-344.
[37] P. Furió-Tarí, S. Tarazona, T. Gabaldon, A.J. Enright, A. Conesa. (2016). spongeScan: A web for detecting microRNA binding elements in lncRNA sequences. Nucleic acids research. 44(W1): W176-W180.
[38] T.B. Hansen, T.I. Jensen, B.H. Clausen, J.B. Bramsen, B. Finsen, C.K. Damgaard, J. Kjems. (2013). Natural RNA circles function as efficient microRNA sponges. Nature. 495(7441): 384-388.
[39] A. Dhir, S. Dhir, N.J. Proudfoot, C.L. Jopling. (2015). Microprocessor mediates transcriptional termination of long noncoding RNA transcripts hosting microRNAs. Nature structural & molecular biology. 22(4): 319-327.
[40] N. Romero-Barrios, M.F. Legascue, M. Benhamed, F. Ariel, M. Crespi. (2018). Splicing regulation by long noncoding RNAs. Nucleic acids research. 46(5): 2169-2184.
[41] P. Johnsson, A. Ackley, L. Vidarsdottir, W.-O. Lui, M. Corcoran, D. Grandér, K.V. Morris. (2013). A pseudogene long-noncoding-RNA network regulates PTEN transcription and translation in human cells. Nature structural & molecular biology. 20(4): 440-446.
[42] S.-M. Jeong, S.-Y. Kim, D.-R. Kim, S.-C. Jo, K. Nam, D. Ahn, S.-C. Lee. (2004). Effect of heat treatment on the antioxidant activity of extracts from citrus peels. Journal of agricultural and food chemistry. 52(11): 3389-3393.
[43] A. Salviano-Silva, S.C. Lobo-Alves, R.C.d. Almeida, D. Malheiros, M.L. Petzl-Erler. (2018). Besides pathology: long non-coding RNA in cell and tissue homeostasis. Non-coding RNA. 4(1): 3.
[44] J.L. Rinn, M. Kertesz, J.K. Wang, S.L. Squazzo, X. Xu, S.A. Brugmann, L.H. Goodnough, J.A. Helms, P.J. Farnham, E. Segal. (2007). Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell. 129(7): 1311-1323.
[45] R.A. Gupta, N. Shah, K.C. Wang, J. Kim, H.M. Horlings, D.J. Wong, M.-C. Tsai, T. Hung, P. Argani, J.L. Rinn. (2010). Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature. 464(7291): 1071-1076.
[46] H. Zhong, S. Liu, Y. Wang, D. Xu, M. Li, Y. Zhao, X. Zeng. (2022). Primary Sjögren's syndrome is associated with increased risk of malignancies besides lymphoma: a systematic review and meta-analysis. Autoimmunity Reviews. 21(5): 103084.
[47] S. He, S. Liu, H. Zhu. (2011). The sequence, structure and evolutionary features of HOTAIR in mammals. BMC evolutionary biology. 11: 102.
[48] L. Lu, G. Zhu, C. Zhang, Q. Deng, D. Katsaros, S.T. Mayne, H.A. Risch, L. Mu, E.M. Canuto, G. Gregori. (2012). Association of large noncoding RNA HOTAIR expression and its downstream intergenic CpG island methylation with survival in breast cancer. Breast cancer research and treatment. 136(3): 875-883.
[49] J.-J. Zhou, D. Cheng, X.-Y. He, Z. Meng, W.-Z. Li, R.-F. Chen. (2017). Knockdown of Hotair suppresses proliferation and cell cycle progression in hepatocellular carcinoma cell by downregulating CCND1 expression. Molecular medicine reports. 16(4): 4980-4986.
[50] X. Zeng, S. Chen, H. Huang. (2011). Phosphorylation of EZH2 by CDK1 and CDK2: a possible regulatory mechanism of transmission of the H3K27me3 epigenetic mark through cell divisions. Cell cycle. 10(4): 579-583.
[51] K. Kim, I. Jutooru, G. Chadalapaka, G. Johnson, J. Frank, R. Burghardt, S. Kim, S. Safe. (2013).

HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer. Oncogene. 32(13): 1616-1625.

[52] E. Li, Z. Zhao, B. Ma, J. Zhang. (2017). Long noncoding RNA HOTAIR promotes the proliferation and metastasis of osteosarcoma cells through the AKT/mTOR signaling pathway. Experimental and therapeutic medicine. 14(6): 5321-5328.

[53] Y. Liu, F. Luo, Y. Xu, B. Wang, Y. Zhao, W. Xu, L. Shi, X. Lu, Q. Liu. (2015). Epithelial-mesenchymal transition and cancer stem cells, mediated by a long non-coding RNA, HOTAIR, are involved in cell malignant transformation induced by cigarette smoke extract. Toxicology and Applied Pharmacology. 282(1): 9-19.

[54] X. Yang, E. Luo, X. Liu, B. Han, X. Yu, X. Peng. (2016). Delphinidin-3-glucoside suppresses breast carcinogenesis by inactivating the Akt/HOTAIR signaling pathway. BMC cancer. 16: 1-8.