lincRNA HOTAIR as a Novel Promoter of Cancer Progression

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Abstract: Large intergenic non-coding RNAs (lincRNA) regulate development and disease *via* interactions with their protein partners. Expression of the lincRNA HOX transcript antisense RNA (HOTAIR) is elevated in a variety of malignancies and linked to metastasis and poor prognosis. HOTAIR promotes proliferation, invasion, and metastasis in the preclinical studies of cancer through modulation of chromatin modifying complexes. In the current review we discuss the molecular mechanisms of HOTAIR-mediated aggressive phenotypes of cancer, HOTAIR's potential in cancer intervention, and challenges in exploration of HOTAIR in cancer biology.

Keywords: lincRNA, cancer, PRC2, EZH2, metastasis.

INTRODUCTION

A groundbreaking discovery in the transcriptome research is that the majority of the human genome is actively transcribed although only ~4% of the transcribed RNAs are translated into proteins [1, 2].One family of the non-coding RNAs is operationally defined as long non-coding RNAs (lncRNA) based on a length > 200 nucleotides [3]. One predominant mode of action of lncRNAsis to govern the expression of protein-coding genes and thereby to regulate development and disease [4]. A global survey of lncRNAs and their co-regulated protein-coding genes has tied lncRNAs to the pathways pivotal to cancer [5].

Large intergenic non-coding RNAs (lincRNA), a subset oflncRNAs, are expressed from a genomic locus between the protein-coding loci [2, 5]. Thousands of lincRNAs have been identified in the human and the mouse [5, 6]. The importance of lincRNA genes are indicated by their global properties, such as a tendency for locations next to developmental regulators, enrichment of tissue-specific expression patterns, high conservation among species, and frequent association with genetic traits [2]. For instance, dozens of lincRNAs are essential to maintenance of the pluripotent state of embryonic stem cells [7]. The expression of these lincRNA genes are regulated by the key transcription factors in embryonic stem cells and their transcripts in turn regulate the gene expression program of pluripotency. lincRNAs govern development and disease *via* regulation of fundamental biochemical and cellular processes, such as gene expression, RNA splicing, and ligand-receptor engagement [8]. One paradigm of lincRNA-mediated regulation of gene expression is that lincRNAs act as a recruiter and scaffold for assembly of chromatin modifying complexes on their target genes [9-13]. Numerous lincRNAsare linked to cancer as well as other human diseases such as facioscapulohumeral muscular dystrophy and Prader-Willi syndrome [14]. In the current review we focus on HOX transcript antisense RNA (HOTAIR), atumor-promotinglincRNA [15].

Discovery of the HOTAIR Gene

HOTAIR was discovered by Rinn and his colleagues as a lincRNA that marks the homeobox D gene cluster (HOXD) for transcriptional repression [15]. The human HOTAIR gene (Ensembl ID: ENSG00000228630) is located on the opposite strand within the intergenic region between HOXC11 and HOXC12 on chromosome 12.The orthologs of the human HOTAIR gene exist only in mammals and appear to evolve faster than its neighboring HOX genes [16, 17]. The human HOTAIR gene is transcribed into 5 variants. Its major transcript (Ensembl ID: ENST00000424518) is 2,421 nucleotide

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long, composed of 7 exons, spliced, and polyadenylated. Its 5' domain (300 nucleotides) binds to the polycomb repressive complex 2 complex (PRC2) and its 3' domain (646 nucleotides) binds to the lysine-specific demethylase 1 (LSD1) complex [11]. HOTAIR acts as a molecular scaffold for assembly of a histone modifying complex consisting of PCR2 and LSD1and recruits the complex to its target genes for transcriptional repression [11]. The PRC2 complex containsa histone methyltransferase named enhancer of zeste homolog 2 (EZH2) that marks a gene for transcriptional repression *via* tri-methylation of histone H3 Lys27 (H3K27me3) [18]. The LSD1 complex possesses histone demethylase activity and marks a gene for transcriptional repression *via* demethylation of the tri-methylated histone H3 Lys4 (H3K4me3), a histone code for transcriptional activation [19]. It is plausible that HOTAIR achieves maximal repression by coupling an increase of the repression code H3K27me3 (PRC2-mediated methylation) with a decrease of the activation code H3K4me3 (LSD1-mediateddemethylation). In accordance deletion of the mouse HOTAIR gene results in de-repression of the HOXD cluster genes, which correlates with concurrent decreased occupancy of H3K27me3 and increased occupancy of H3K4me3 on those genes [20]. As a consequence HOTAIR null mice exhibit homeotic transformation of the spine and malformation of metacarpal bones [20].

Expression of HOTAIR in Cancer

Since its emergencein breast cancer, elevated expression of HOTAIR has been reported in 16 types of malignancies [9, 21-51] (Table **1**). The spectrum ranges from carcinomas of epithelial origin to gastrointestinal stromal tumor of stromal origin [9, 43, 52]. Surprisingly dysregulated expression of HOTAIR has not been reported in hematological malignancies in which the HOX genes are frequently dysregulated [53, 54]. An intriguing phenomenon in breast cancer is that the established tumor cell lines exhibit a much lower expression of HOTAIR than the tumor tissues [9, 21-51].This apparent discrepancy might be attributed to activation of HOTAIR expression by several invasion-promoting cues that are aberrantly enriched in the tumor micro environment but absent in routine culture condition. For instance addition of transforming growth factor-β1 to culture activates the expression of HOTAIR in breast and colon cancer cells [21]. In addition prolonged exposure of human breast cancer MCF-7 cells to tumor necrosis factor-α results in a robust increase in HOTAIR expression (unpublished observations). Moreover type 1 collagen transcriptionally up-regulates the expression of HOTAIR in lung adenocarcinoma cells [50]. Interestingly, those stimuli are potent inducers of epithelial-mesenchymal transition (EMT) and can up-regulate expression of another tumor-promoting non-coding RNA miR-21, which strengthens a mechanistic link between non-coding RNAs and invasiveness [55-59]. In human breast cancer MCF-7 cells the expression of HOTAIR is activated by estradiol through recruitment of a transcriptional co-activator mixed lineage leukemia (MLL) that marks the HOTAIR promoter for activation *via*H3K4me3 [22]. HOTAIR expression is also induced by bleomycin and radiation in human breast cancer MCF-7 cells, which implies a role for HOTAIR in cell response to DNA damage [44]. A recent report has revealed a novel layer of regulation of HOTAIR expression [25]. The HOTAIR transcript harbors a seed sequence (GenBank NR_047517, position 902-923) for the tumor suppressive microRNA miR-34a [25, 60, 61]. miR-34a suppresses the expression of a reporter gene that is controlled by the miR-34a seed sequence identified in the HOTAIR transcript [25]. This phenomenon, if proven to be widespread, establishes a novel yet critical interaction between two major non-coding regulators of cancer, microRNAs and lncRNAs [62, 63]. Taken together the expression of HOTAIR is regulated by the tumor modulating cues.

Function of HOTAIR in Cancer

Compelling evidence links elevated expression of HOTAIR to metastasis and poor prognosis in a variety of tumors (Table **1**). Elevated expression of HOTAIR reprograms PCR2-mediated gene repression, which results in a shift from repression of tumorigenic genes to repression of tumor suppressive genes [9, 30, 32, 33]. As a consequence, HOTAIR regulates several signaling pathways that are pivotal to invasive and proliferative phenotypes. EMT leads to invasiveness and stemness of cancer cells [64]. Induction of HOTAIR by EMT inducers is required for EMT because knockdown of HOTAIR by RNAi abrogates the expression of EMT markers and invasion in cancer stem cells [21, 48]. Epigenetic regulation accounts for the profound alteration in gene expression during EMT [65]. Over-expression of HOTAIR reprograms gene expression to promote invasion *via* its interaction with PRC2 and consequent H3K27me3 on its target genes [9]. It is plausible that HOTAIR is up-regulated by EMT inducers and such an induction in turn promotes the gene expression program that mediates EMT

Table 1: Malignancies Associated with Elevated Expression of HOTAIR

Types	Interacting molecules/pathways	Cellular Processes	Clinical manifestations
Ameloblastomas			Keratinized phenotype [45]
Breast cancer	BRCA1 [47] Estrogen [22] EMT/Stemness [21] DNA methylation [38] PRC2 [9]	Invasion [9] Proliferation & apoptosis [22] Genotoxic stress [44]	Metastasis [9, 24] ER and PR positivity [24] Poor survival [9, 24]
Colorectal cancer	EMT/Stemness [21] PRC2 [33]	Invasion [21]	Metastasis, poor prognosis [33]
Esophageal cancer	Wnt/β-catenin [28]	Proliferation, invasion, apoptosis, metastasis [23, 35, 39]	Advanced stage, poor survival [23, 28, 39]
Gastric cancer	PRC2 [30] EMT [48]	Proliferation, invasion, apoptosis, metastasis [27, 48]	Lymph node metastasis, advanced TNM staging, poor survival [27, 30]
GIST		Invasion [43]	High risk grade, metastasis [43]
Glioma		Cell cycle progression [43]	Poor prognosis [43]
HCC	Gelatinase, VEGF [29]	Invasion [29, 49] Proliferation [31] Apoptosis, chemosensitivity [49]	Lymph node metastasis, recurrence [29, 49] Poor prognosis [31, 49]
Laryngeal cancer	PTEN [34]	Invasion, apoptosis [34]	Advanced stages, poor prognosis [34]
Lung cancer	HOXA5 [36] Col-1 [50] p21cip1/waf1 [37]	Morphogenesis [50], Invasion [36], Cisplatin resistance, cell cycle, apoptosis [37]	Lymph node metastasis, poor prognosis [36] Brain metastasis [41]
Melanoma	Gelatinase [46]	Invasion [46]	Lymph node metastasis [46]
NPC		Proliferation, invasion [42]	Lymph node metastasis, poor survival [42]
Ovarian cancer			Advanced stage, poor differentiated [26]
Pancreatic cancer	PRC2 [32]	Proliferation, apoptosis [32]	Advanced stage [32]
Prostate cancer	miR-34a [25]	Proliferation, invasion, apoptosis, castration resistance [25]	
Sarcoma			Metastasis, therapeutic resistance [40]

GIST: gastrointestinal stromal tumor; HCC: hepatocellular carcinoma; NPC: nasopharyngeal carcinoma; EMT: epithelial-mesenchymal transition; ER: estrogen receptor; PR: progesterone receptor; PRC2: polycomb repressive complex 2; PTEN: Phosphatase and tensin homolog.

[21, 48, 50]. For instance HOTAIR represses the expression of Wnt inhibitory factor 1 (WIF-1), an inhibitor of the Wnt/β-catenin pathway that mediates EMT in cancer [28, 66]. In addition HOTAIR represses the expression of phosphatase and tensin homolog(PTEN), an inhibitor of EMT [34, 67]. HOTAIR also regulates the effectors of invasion as HOTAIR is required for the expression of matrix metalloproteinases that break down extracellular matrix to pave the path for invasion [29, 46, 48]. In consistence with these *in vitro* studies, HOTAIR is required for metastasis of grafted cancer cells in mice [9, 27, 35, 36]. Besides invasion and metastasis, elevated expression of HOTAIR is essential to proliferation and resistance to apoptosis in a variety of cancer cells (see Table **1**). The pro-proliferative and pro-survival actions of HOTAIRcan be attributed to the HOTAIR-mediated suppression of the anti-proliferative genes (i.e., p21cip1/waf1) and the anti-survival genes (i.e., PTEN) [34, 37].

Diagnostic and Therapeutic Potential of HOTAIR in Cancer

Because of the elevated expression of HOTAIR is observed in the malignant tissues over their adjacent normal tissues and correlated with metastasis and poor

prognosis, HOTAIR can be explored as a biomarker for metastasis of a variety of tumors (Table **1**). In addition HOTAIR is a potential biomarker for patients' response to certain anti-cancer treatments because HOTAIR is linked to resistance to cisplatin in lung cancer cells and resistance to castration in prostate cancer cells [25, 37]. Its feasibilityas a biomarker is affirmed by the findings that lincRNAs are stable and measurable in body fluids and thereby suitable for measurement *via* non-invasive procedures [68]. For instance, HOTAIR along with several other lincRNAs can be detected and quantitated in plasma samples collected from the patients with gastric cancer [69].

HOTAIR is a promising therapeutic target because HOTAIR promotes proliferation, survival, and invasion in cancer cells (Table **1**). In support of HOTAIR's therapeutic potential, inhibitors of EZH2, the catalytic subunit of PRC2 that HOTAIR directly binds to, has emerged as a promising anti-cancer drug in pre-clinical studies as well as early phases of clinical trials [70, 71]. It is appealing to selectively disrupt the interaction between HOTAIR and EZH2 in cancer cells upon successful molecular and biochemical resolution of HOTAIR's binding to EZH2. In addition to disruption of the HOTAIR-EZH2 interaction, therapeutic targeting of HOTAIR can come at a number of other levels. Base-pairing driven oligonucleotide-based methods are particularly attractive against lncRNAs [72]. For instance, small interfering RNA (siRNA) against HOTAIR has been shown to attenuate invasiveness in breast and gastric cancer cells as well as growth of pancreatic cancer in a mouse xenograft model [9, 32, 48]. Further, synthetic single stranded antisense DNA is effective at knocking down HOTAIR and leading to apoptosis in MCF7 breast cancer cells [22]. Importantly, a number of complementary therapeutic strategies are concurrently being developed to target mRNA and microRNA (such as using ribozymes, aptamers, and small molecules that selectively bind to target RNAs) [72]. As the structural information of HOTAIR is further elucidated these methods could be readily applied to inhibit HOTAIR.

CHALLENGES AND FUTURE DIRECTIONS

HOTAIR has emerged as an appealing diagnostic and therapeutic target for cancer. However several challenges hinder fulfillment of HOTAIR's potential in cancer care. One challenge is our limited understanding of the interaction between HOTAIR and EZH2 although a 300 nucleotide binding domain is identified in HOTAIR [11]. A high resolution mapping of HOTAIR-EZH2 binding is essential to develop compounds that can effectively and specifically disrupt the HOTAIR-EZH2 interaction in cancer cells. This need is demanded by the fact that the EZH2-containing PRC2 physically interacts with thousands of lincRNAs and its function is tightly controlled by the bound lincRNAs [6, 73]. It is conceivable that PRC2 can form a pool of hundreds of functional units as defined by their lincRNA partners and the composition of those units is dynamically fine-tuned to maintain an appropriate gene expression program to a cell's needs in a particular cellular context. How an increased expression of HOTAIR impairs this fine-tuned pool of PRC2-lincRNA units and promotes cancer is a daunting yet important question to answer [74]. Another question arises from EZH2-mediated methylation of non-histone proteins, such as transcription factor GATA4 and consequent regulation of the GATA4-mediated gene expression [75]. Undoubtedly inhibition of either HOTAIR or EZH2 reduces tumor progression [9] (also see Table **1**). However the experimental approaches used in those studies are not able to exclude the possibility that the altered gene expression and cell behaviors can be, at least in part, attributed to altered methylation of the transcription factors and other non-histone proteins methylated by EZH2. It is also naive to conclude that PRC2 and LSD1 are the sole protein partners of HOTAIR and epigenetic regulation of gene expression is the sole function of HOTAIR. lincRNAs have been localized in every subcellular compartments and linked to a wide range of cell functions, such as signaling transduction, RNA splicing, ligand-receptor engagement [8]. One such exemplary is metastasis associated lung adenocarcinoma transcript 1 (MALAT1), a tumor associated lincRNA that regulates gene expression, RNA splicing, and formation of nuclear speckles *via* distinct mechanisms [76]. A versatility of HOTAIR function in cancer can be explored upon a successful probing of HOTAIR-bound protein partners using HOTAIR as a bait in cancer cells. Despite these challenges HOTAIR is a novel and promising target in diagnostic and therapeutic interventions of cancer.

ABBREVIATIONS

lincRNA = long intergenic non-coding RNA

HOTAIR = HOX transcript antisense RNA

PRC2 = polycomb repressive complex 2

EZH2 = enhancer of zeste homolog 2

LSD1 = lysine-specific demethylase 1

PTEN = Phosphatase and tensin homolog

COMPETING INTERESTS

The authors declare no conflict of interests.

AUTHORS' CONTRIBUTION

All authors have contributed to the preparation of this manuscript. All authors have read and approved the manuscript.

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