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Mini-review

HOX transcript antisense RNA (HOTAIR) in cancer

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ABSTRACT

Long noncoding RNAs (lncRNAs) have emerged as a new family of master regulators of cancer. The lncRNA HOX transcript antisense RNA (*HOTAIR*) is a prime example of an oncogenic *trans*-acting lncRNA. The expression of *HOTAIR* is elevated in a broad spectrum of cancers and is associated with metastasis and poor prognosis. *HOTAIR* governs fundamental biochemical and cellular processes via interactions with a variety of partners to promote proliferation, invasion, survival, drug resistance, and metastasis in preclinical studies of cancer. Here, we review the diagnostic and therapeutic potential of *HOTAIR* as well as the molecular mechanisms underlying the dysregulation of its expression and function in cancer. We also discuss the challenges to capitalizing on *HOTAIR* for more effective patient care along with future directions founded on the recent exciting advances in our knowledge of *HOTAIR* in cancer.

1. Introduction

Recent advances in analyzing the human transcriptome have revealed that ~90% of the human genome is actively transcribed, although only ~3% encodes proteins [1]. Transcribed RNAs devoid of protein-coding potential are called noncoding RNAs. One such family of noncoding RNAs is operationally defined as long non-coding RNAs (lncRNAs) based on their length of > 200 nucleotides [2]. Thus far, the lncRNA catalogue comprises more than 10,000 manually annotated lncRNA genes that produce more than 15,000 lncRNAs [1]. The lncRNA family is heterogeneous and can be classified by position (*e.g.* long intergenic RNAs), structure (*e.g.* circular RNAs), function (*e.g.* competing endogenous RNAs), and orientation of transcription (*e.g.* antisense RNAs). Similar to mRNAs, the majority of lncRNAs are transcribed by RNA polymerase II, 5′-capped, 3′-polyadenylated, and spliced. lncRNAs have emerged as master regulators of fundamental molecular and cellular processes, such as chromatin remodeling during transcription, RNA splicing, and ligand-receptor engagement [3]. Moreover, numerous lncRNAs have been defined as oncogenes and tumor suppressors in a wide variety of solid tumors and hematological malignancies [4]. HOX transcript antisense RNA (*HOTAIR*) is a prime example of an oncogenic lncRNA. Elevated expression of *HOTAIR* in cancer was first discovered in breast cancer and is associated with poor survival and metastasis [5]. *HOTAIR* reprograms global chromatin state to promote invasion and metastasis via its recruitment of polycomb repressive complex 2 (PRC2) to its target genes in breast cancer cells [5]. Since this discovery, *HOTAIR* has attracted intense investigation in the field of cancer. A search for *HOTAIR* and cancer as keywords in the title/abstract yielded 515 articles in PubMed as of November 2018. The functions of *HOTAIR* in cancer have also been critically summarized and reviewed recently [6,7]. These reviews highlight a critical role for *HOTAIR* in tumor growth, apoptosis, invasion, metastasis, cancer stem cell differentiation, and drug resistance.

HOTAIR was discovered by Howard Chang's group as a *trans*-acting intergenic lncRNA that is transcribed from the homeobox gene C cluster (*HOXC*) and recruits PRC2 to repress transcription from the *HOXD* cluster in fibroblasts [8]. Its canonical 6 exon transcript, *HOTAIR-C* (RefSeq NR_003716), is a 2364 bp RNA transcribed from a 6449 bp gene locus that is intergenic and antisense with respect to *HOXC11* and *HOXC12*. Besides its canonical isoform, the RefSeq catalogue includes two other HOTAIR isoforms: *HOTAIR-U* (NR_047518) and *HOTAIR-N* (NR_047517) (Fig. 1). *HOTAIR-N* is distinct from the two other isoforms in that its transcription is initiated from the first intron of *HOXC11* in an antisense fashion and its unique first exon and intron overlap with *HOXC11*.

Pioneering studies have identified several critical protein partners for *HOTAIR*'s functions (Fig. 2). The most studied partner is PRC2, a protein that marks a gene for transcriptional repression via

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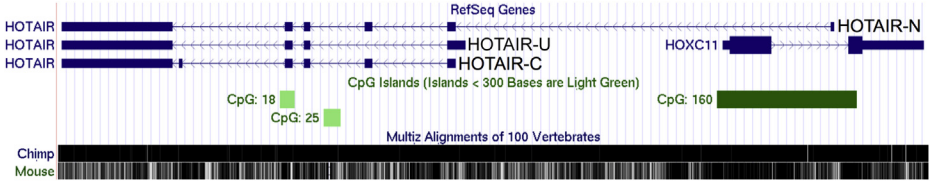

Fig. 1. Human *HOTAIR* isoforms. A screenshot of the human *HOTAIR* isoforms in RefSeq using the UCSC Genome Browser. *HOTAIR-N* corresponds to NR_047517. *HOTAIR-C* corresponds to NR_003716. *HOTAIR-U* corresponds to NR_047518. The dark green rectangle represents the CpG island that resides in the *HOTAIR-N* and *HOXC11* overlapping region and consists of 160 CpG sites.

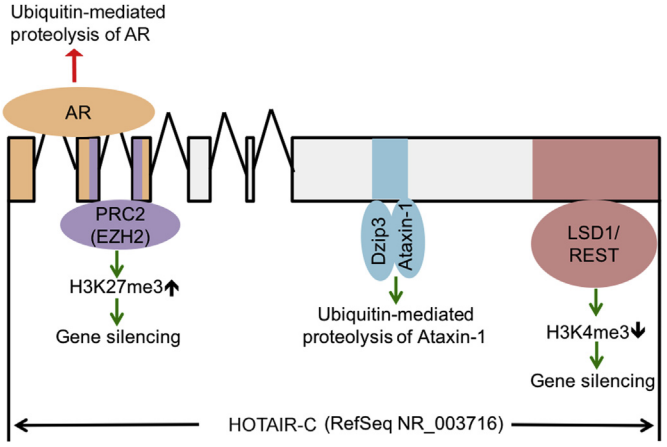

Fig. 2. Protein partners of *HOTAIR*. The interactions between *HOTAIR* and its protein partners are summarized. The lengths of each exon and the positions of the interacting regions are proportional to their lengths and positions in the canonical transcript, *HOTAIR-C* (RefSeq NR_003716). The introns are not drawn in proportion to their length. Green arrows indicate positive regulation of the targeted processes. Red arrows indicate negative regulation of the targeted processes. PRC2: polycomb repressive complex 2; EZH2: enhancer of zeste homolog 2; AR: androgen receptor; HuR: human antigen R; LSD1: lysine-specific demethylase 1.

trimethylation of histone H3 Lys 27 (H3K27me3) [9]. *HOTAIR* binds to GA-rich motifs in the genome to nucleate broad domains of PRC2 occupancy that lead to PRC2-mediated H3K27me3 and gene repression [10]. PRC2 interacts with an 89 bp fragment in the 5′ end of *HOTAIR* (Fig. 2) [8]. However, an alternative mechanism for the interaction between *HOTAIR* and PRC2 has been proposed in which the short repeats of consecutive guanines in *HOTAIR*, rather than a specific structural domain, may be responsible for the interaction with PRC2 [11]. Two recent reports support this concept by showing that RNA G-quadruplexes (G4s) in the 5′ region (1–400 bp) of *HOTAIR* mediate the interaction with PRC2 [11,12]. RNA G4s are formed by stacks of two or more connected square planes consisting of 4 guanines that are stabilized via Hoogsteen hydrogen bonding interactions and are implicated in many essential cellular processes and the pathogenesis of many diseases, including cancer [13]. Indeed, the 5′ 400 bp region of *HOTAIR* is enriched with tracts of Gs, and two overlapping RNA G4s (281–340 bp & 311–370 bp) have been identified using G4 RNA Screener 2.0 [14]. Another critical partner of *HOTAIR* is the LSD1/CoREST/REST complex, which contains lysine-specific demethylase 1 (LSD1) [15]. LSD1 is a histone demethylase that represses gene expression by reducing the trimethylation of histone H3 Lys 4 (H3K4me3), a marker of gene activation. The LSD1 complex binds to *HOTAIR* via a 646 bp fragment in the last exon of the lncRNA (Fig. 2). Taken together, *HOTAIR* acts as a molecular scaffold for the assembly of a repressor complex consisting of PRC2 and LSD1, thereby coupling increased H3K27me3 with decreased H3K4me3 on target genes to efficiently repress gene expression [16]. In addition to chromatin modifications, *HOTAIR* can also facilitate ubiquitin-mediated proteolysis via its physical interactions with two E3 ubiquitin ligases, Dzip3 and Mex3b (Fig. 2) [17]. In contrast, binding of *HOTAIR*, via its 5′ 1–360 bp region, to androgen receptor (AR) prevents ubiquitin-mediated degradation of AR (Fig. 2) [18]. Lastly, *HOTAIR* can act as a competitive endogenous RNA sponge for dozens of miRNAs, as reviewed recently [19].

Due to the broad range of functions of *HOTAIR* in the regulation of gene expression and protein proteolysis, this lncRNA has been associated with many different types of cancers. Herein, we review the recent advances regarding the role of *HOTAIR* in cancer and discuss the unanswered critical questions related to how dysregulation of *HOTAIR* contributes to tumor initiation and progression.

2. Clinical manifestation and diagnostic/therapeutic potential associated with *HOTAIR* upregulation in cancer

Dysregulated *HOTAIR* expression and function have been reported in at least 24 types of solid tumors (Table 1). In general, elevated expression of *HOTAIR* is associated with shorter overall survival and disease free survival, metastasis, and resistance to chemo/radiotherapy in cancer (Table 1) [5,18,20–40,41–60,61–89]. Indeed, *HOTAIR* has been shown to promote proliferation, survival, stemness, invasion, and resistance to therapy via interactions with molecules such as chromatin modifiers, ubiquitin ligases, and miRNAs (Table 1). These findings suggest that elevated expression of *HOTAIR* is a biomarker for cancer diagnosis, metastasis, resistance to therapy, and poor survival. The promise of *HOTAIR* as a biomarker is strengthened by the fact that *HOTAIR* is stable and measurable in body fluids, with its increase bearing diagnostic and prognostic values in gastric cancer, melanoma, pancreatic cancer, cervical cancer, breast cancer, and glioblastoma [52,72,94–97].

HOTAIR is also a promising therapeutic target because it promotes proliferation, survival, and invasion in a variety of cancer cells (Table 1). Accordingly, *HOTAIR* antisense oligonucleotides have demonstrated therapeutic potential in preclinical studies. For instance, a small interfering RNA (siRNA) against *HOTAIR* has been shown to abolish the growth and invasion of breast, pancreatic, and gastric cancer cells *in vitro* and *in vivo* [91,98–100]. Furthermore, it is appealing to selectively disrupt the interactions between *HOTAIR* and its partners to abrogate their oncogenic activities. For example, a peptide nucleic acid hybrid was designed to block *HOTAIR* binding to EZH2 in order to re-sensitize chemo-resistant ovarian tumors to platinum [101]. Therefore, it is conceivable to expand the utilization of this concept to disrupt *HOTAIR*'s interactions with other partners in cancer cells.

Realization of the diagnostic and therapeutic potential of *HOTAIR* demands a thorough understanding of how *HOTAIR*'s expression and function are dysregulated in cancer cells. Therefore, we will review the mechanisms underlying the dysregulation of *HOTAIR* in cancer in the following sections.

3. Upregulation of *HOTAIR* in cancer

Unlike most classical oncogenes, the *HOTAIR* gene is rarely amplified or mutated in cancer, as revealed by a survey of sequencing data from The Cancer Genome Atlas (TCGA) using cBioportal [102]. Thus, transcriptional upregulation of *HOTAIR* likely underlies its increased expression in cancer cells. Interestingly, a cancer risk-associated SNP, rs920778, has been identified in an enhancer-like region located between 1719 bp and 2353 bp downstream of *HOTAIR-C*'s transcription start site in intron 2 [103]. The rs920778T allele is correlated with higher expression of *HOTAIR* in esophageal tissue [103]. Moreover, the

Table 1
Malignancies Associated with Elevated Expression of HOTAIR. 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.

Types	Interacting molecules/pathways	Cellular Processes	Clinical manifestations
Bladder cancer	miR-205 & cyclin J [20]	Proliferation, invasion & migration [20]	Recurrence and survival [21]
Breast cancer	BRCA1 [22] ER [23–25] PRC2 [5] TGF-β1 [26]	Invasion & metastasis [5] Proliferation & apoptosis [90] EMT & Stemness [26,27] DNA methylation [28] Tamoxifen resistance [25]	Metastasis [5] ER and PR positivity [29] Poor survival [5] Response to endocrine therapy [24]
Cervical Cancer	Wnt [30] HIF1-A [31] miR-143-3p & BCL-2 [32] NOTCH [33]	Autophagy [30] Radioresistance [30,31] Cell proliferation and apoptosis [31] EMT and Invasion [33]	Tumor size, lymph node metastasis, and survival [33]
Colorectal cancer	PRC2 [34] miR-203a-3p & Wnt/ß-Catenin [35]	EMT/Stemness & invasion [27] Chemoresistance [35]	Metastasis, poor prognosis [34]
Endometrial cancer	miR-646-NPM1 axis [36]	Estrogen induced invasion & metastasis [36]	Metastasis and poor survival [37]
Esophageal cancer	Wnt/β-catenin [38] miR-1 & CCND1 [39]	Proliferation, invasion, apoptosis, metastasis [39–41]	Advanced stage, poor survival [39–41]
Gall bladder cancer	miR-130a [42]	Invasion and proliferation [42]	
Gastric cancer	PRC2 [43] miR-217 [44]	Proliferation, invasion, apoptosis, metastasis [45,46] EMT [46] Chemoresistance [44]	Lymph node metastasis, advanced TNM staging, poor survival [43,45]
GIST		DNA methylation [47], Invasion [48]	High risk grade, metastasis [48]
Glioma	miR-15b [49] miR-126-5p [50]	Proliferation [49]	Poor prognosis [51,52]
HCC	Gelatinase, VEGF [53] miR-23b-3p [54] DDX5 [55] SETD2 [56]	Invasion [53,57] Proliferation [58] Apoptosis, chemosensitivity [57] EMT [54] Cancer stemness [59]	Lymph node metastasis, recurrence, Poor prognosis [53,57]
HNSCC	STAT3 [60]	Proliferation & chemosensitivity [61]	Poor overall survival, Lymph node metastasis [62]
Laryngeal cancer	PTEN [63]	Invasion, apoptosis [63]	Advanced stages, poor prognosis [63]
Lung cancer	HOXA5 [64] p21cip1/waf1 [65]EZH2 [66] FOXA1, FOXA2, LSH [67]	Morphogenesis [68], Invasion [69], Cisplatin resistance, cell cycle, apoptosis [65,70]	Lymph node metastasis, poor prognosis [64]
Melanoma	Gelatinase [71]	Invasion [71]	Lymph node metastasis [72]
Neuroblastoma			Polymorphism linked to increased risk [73]
NPC	VEGFA [74]	Proliferation, invasion [75] Angiogenesis [74]	Lymph node metastasis, poor survival [76]
Oral Carcinoma	PRC2 [77–79]	EMT, cancer stemness, and metastasis [80] Proliferation and apoptosis [79]	Overall survival [79]
Ovarian cancer	miR-206 [81] NF-KB [82]	Proliferation [81] Cell senescence, Chemoresistance [82]	Advanced stage, poor differentiated [83] Platinum resistance [82]
Pancreatic cancer	PRC2 [91] miR-34a & EZH2 [92]	Proliferation, apoptosis [91,92]	Advanced stage [91]
Prostate cancer	miR-34a [84] Androgen receptor [18]	Proliferation, invasion, apoptosis, castration resistance [18,93]	castration-resistant prostate cancer [18,93]
Renal Cell Carcinoma	miR-203 [85] IGFBP2 [86]	EMT [85]Migration [86]	nuclear grade, lymph-node metastasis, and lung metastasis [86]
Osteosarcoma	miR-454-3p [87]	Invasion [88]	Metastasis, therapeutic resistance [88]
Urothelial Carcinoma		Differentiation and aggressiveness [89]	Elevated expression [89]

rs920778T allele-containing enhancer drives higher expression of a reporter gene than the rs920778C allele-containing enhancer [103].

Most investigations of the upregulation of *HOTAIR* in cancer have been focused on the regulatory regions surrounding the *HOTAIR-C* transcription start site. As a critical component in the oncogenic network, it is not surprising that *HOTAIR* is transcriptionally activated by classical oncogenes. For instance, transcription of *HOTAIR* is activated by Myc through an E-box located 1053 bp upstream of *HOTAIR-C*'s transcription start site in gallbladder cancer cells [42]. Moreover, NF-κB-responsive elements have been identified in the promoter region upstream of *HOTAIR-C* in ovarian cancer cells [82]. In breast cancer cells, activated estrogen receptor binds the *HOTAIR-C* promoter and activates *HOTAIR* expression via the recruitment of mixed lineage leukemia proteins (MLLs), histone methyltransferases that mark *HOTAIR-C*'s promoter for transcription via H3K4me3 [23].

Besides its promoter region, regulatory elements have also been identified downstream of the 3′ end of the *HOTAIR* gene. An intriguing observation in breast cancer is that increased expression of *HOTAIR* correlates with increased DNA methylation in an intergenic CpG island located between *HOTAIR* and *HOXC12* [28]. It is proposed by the authors of this study that this hypermethylated intergenic CpG island serves as a barrier to prevent repressive heterochromatin from spreading from the neighboring *HOXC12* gene into the *HOTAIR* gene [28]. Another report revealed a distal enhancer region downstream of the 3' end of *HOTAIR* that acts from long range to upregulate *HOTAIR* expression via DNA looping in breast cancer [24]. Importantly, this distal enhancer-mediated activation of *HOTAIR* plays a critical role in mesenchymal to epithelial transition because hepatocyte nuclear factor 4-α, an inducer of epithelial differentiation, directly represses *HOTAIR* transcription by disrupting the chromatin loop between *HOTAIR* and the distal enhancer [104].

The expression of *HOTAIR* can be stimulated by extrinsic cues commonly found in the tumor microenvironment. These extrinsic cues are worthy of particular consideration in preclinical research of *HOTAIR* because established breast cancer cell lines exhibit a much lower expression of *HOTAIR* than primary human breast cancer tissues [5]. This apparent discrepancy may be attributed to the stimulation of *HOTAIR* expression by tumor-promoting cues that are aberrantly

enriched in the tumor microenvironment, but are absent in routine two-dimensional cell culture. One family of extrinsic cues that are abundant in the tumor microenvironment are inflammation related cytokines. Indeed, transforming growth factor-β1 (TGF-β1) has been shown to induce the expression of *HOTAIR* in breast, colon, and liver cancer cells and this induction is required for the acquisition of epithelial to mesenchymal transition (EMT) and stemness by these cells [26,27,105]. Furthermore, in our previous report, prolonged exposure of human breast cancer MCF-7 cells to tumor necrosis factor-α (TNF-α) induced the expression of *HOTAIR* and promoted EMT [99,106].

Additional extrinsic cues that are present in the tumor microenvironment but are absent in routine cell culture are extracellular matrix (ECM) components. As we previously reported, type 1 collagen, a major component of the fibrotic tumor microenvironment, transcriptionally activates the expression of *HOTAIR* in lung cancer cells in an ECM-based three-dimensional (3-D) organotypic culture [68]. Moreover, our further investigation into the ECM-mediated regulation of *HOTAIR* revealed that *HOTAIR* is induced by laminin-rich ECM in 3-D cultures of claudin-low breast cancer cells, an aggressive molecular subtype of breast cancer [100]. This upregulation of *HOTAIR* is mediated through cell surface ECM receptor integrins and the intracellular signal transducer Src kinase [100]. Our studies also showed that knockdown of *HOTAIR* abrogates invasive growth of claudin-low breast cancer cells in laminin-rich ECM 3-D culture [100]. Overall, the discovery of ECM-induced *HOTAIR* expression in claudin-low breast cancer cells is particularly important because this subtype is enriched with ECM-responsive genes and is refractory to the currently available therapies [107,108].

One critical question that has been largely overlooked is which isoform(s) of *HOTAIR* is/are upregulated in cancer. Answering this question is imperative to successfully silencing *HOTAIR* in cancer cells. The current paradigm is that among *HOTAIR*'s three isoforms, canonical *HOTAIR-C* accounts for the elevated expression of *HOTAIR* in cancer, although most of the previous studies did not examine the expression of each isoform specifically. Interestingly, our initial survey of RNA-SEQ data from the TCGA of paired tumor and non-tumor samples of invasive breast carcinoma suggests that *HOTAIR-N* is actually the predominant isoform in tumor tissues [100]. Similarly, *HOTAIR-N* appears to be the dominant isoform in the TCGA lung adenocarcinoma cohort (unpublished observations). Indeed, *HOTAIR-N* was found to be the highest expressed isoform in 10 out of 12 lung tumor and 10 out of 14 breast tumor samples, respectively. Moreover, *HOTAIR-N* was found to account for 72% and 57% of the total *HOTAIR* expression, on average, in lung and breast tumors, respectively. In contrast, *HOTAIR-C* was found to account for ~26% and ~31%, on average, of the total *HOTAIR* expression in lung and breast tumors, respectively. In our own RNA-SEQ analyses of the human lung cancer cell line H23 and the breast cancer cell line T47D, *HOTAIR-N* was also consistently observed to be the dominant isoform (GSE119513 and GSE119511, unpublished observations).

HOTAIR-N overlaps with *HOXC11*, and the overlapping region includes a strong CpG island (Fig. 1). The head-to-head transcription of *HOTAIR-N* and *HOXC11* through the *HOTAIR-N-HOXC11* CpG island resembles another sense-antisense gene pair, vimentin and *VIM-AS1* [109]. The expression of vimentin and *VIM-AS1* is co-upregulated by an R-loop, a non-B form DNA structure in which the newly synthesized RNA hybridizes with its template DNA strand and causes the non-template G-rich DNA strand to remain looped out in a single-stranded conformation [109,110]. Emerging evidence has linked R-loops to transcription, cytosine methylation, and histone modifications in physiological and pathological conditions because R-loops mediate the expression of vimentin and VIM-AS1 via reducing cytosine methylation in the CpG island in the vimentin and VIM-AS1 gene locus [109]. Interestingly, the *HOTAIR-N-HOXC11* CpG island also harbors R-loop motifs, as revealed by a survey of an R-loop database (Fig. 3A) [111]. Therefore, it is conceivable that activation of *HOTAIR-N* in cancer involves potential interactions between R-loops and epigenetic remodeling, especially reduction of cytosine methylation by R-loop in the *HOTAIR-N-HOXC11* CpG island as illustrated in Fig. 3B.

Besides transcriptional activation, emerging evidence indicates that *HOTAIR* is regulated post-transcriptionally by miRNAs in cancer cells. Indeed, *HOTAIR* harbors a target site for miR-34a in its last exon [84]. miR-34a was found to reduce the expression of *HOTAIR* as well as the expression of a reporter gene controlled by the miR-34a target site from *HOTAIR* in prostate cancer cells. Similarly, a target site for miR-141 has also been identified in the last exon of *HOTAIR* [112]. The miR-141-*HOTAIR* axis is critical to EMT, a process central to invasion and stemness of cancer cells, because miR-141 belongs to the miR-200 family of miRNAs, the most potent miRNA inhibitors of EMT [27,113]. Moreover, *HOTAIR* is targeted by another miRNA tumor suppressor, let-7, which reduces its expression [17]. let-7-mediated decay of *HOTAIR* is achieved through the formation of a hetero-tetramer that consists of *HOTAIR*, let-7, Ago2, and RNA binding protein human antigen R (HuR) [17]. HuR binding to the last exon of *HOTAIR* appears to recruit the let-7/Ago2 complex, targeting HOTAIR for decay [17]. Thus, tumor suppressive miRNAs act in concert to mediate the decay of *HOTAIR*.

4. Molecular and cellular processes regulated by *HOTAIR* in cancer

Elevated expression of *HOTAIR* is correlated with invasion, metastasis, and poor survival (Table 1). One paradigm for the dysregulated function of *HOTAIR* in cancer is that *HOTAIR* binds to PRC2 and reprograms the overall chromatin state, specifically H3K27me3, which consequently shifts repression from oncogenes to tumor suppressors globally [5]. The molecular and cellular processes regulated by *HOTAIR* in cancer are summarized in Table 1. The interaction between *HOTAIR* and PRC2 has drawn the most attention and is well characterized.

One gene that is repressed by *HOTAIR* is $p21^{WAF1/CIP1}$, a protein that mediates p53-induced growth arrest and apoptosis in response to DNA damage [65,114]. In lung adenocarcinoma cells, *HOTAIR* promotes proliferation, survival, and resistance to cisplatin through repression of $p21^{WAF1/CIP1}$ [65,114]. In addition, *HOTAIR* promotes EMT and invasion by repressing the expression of phosphatase and tensin homolog (*PTEN*), an inhibitor of Akt and EMT [63]. *HOTAIR* promotes cytosine methylation of the *PTEN* promoter, but it remains unclear whether *HOTAIR* binding to the *PTEN* promoter directly triggers cytosine methylation or whether cytosine methylation results from *HOTAIR*-mediated H3K27me3. *HOTAIR* also mediates the physical interaction between PRC2 and the transcription factor Snail, a master regulator of EMT [115]. Formation of the tripartite complex recruits PRC2 to the Snail target sites in epithelial marker genes and results in the repression of these genes during EMT [115].

Besides interactions with epigenetic modifiers, *HOTAIR* also interacts with ubiquitin ligases to regulate ubiquitin-mediated proteolysis. The E3 ubiquitin ligase Dzip3 and its substrate ataxin-1 bind in tandem to a ~250 bp region in the last exon of *HOTAIR* through their respective RNA binding domains (Fig. 2) [17]. In addition, the E3 ubiquitin ligase Mex3b and its substrate snurportin-1 bind to *HOTAIR* in two distinct regions: a 125 bp region at the 5' end and a 120 bp region in the last exon, respectively [17]. Through these interactions, *HOTAIR* acts as an assembly scaffold to facilitate the proteolysis of ataxin-1 and snurportin-1 to regulate senescence in cancer cells [17]. In contrast, *HOTAIR* has been reported to shield androgen receptor from E3 ubiquitin ligase MDM2-mediated degradation, thereby enhancing the gene expression program activated by androgen receptor in prostate cancer cells [18].

An emerging theme in cancer is the crosstalk between miRNAs and lncRNAs. As discussed above, the expression of *HOTAIR* is regulated by several tumor suppressive miRNAs, such as miR-141, in cancer cells [112]. On the other hand, *HOTAIR* has also been reported to sequester several tumor suppressive miRNAs via base pairing mechanisms. For

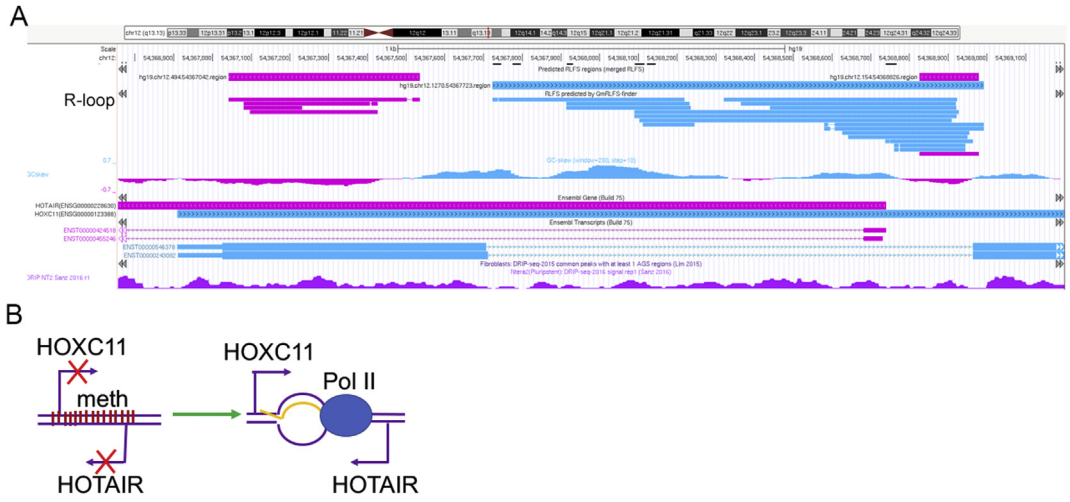

Fig. 3. Illustration of R-loop formation in the *HOTAIR-N-HOXC11* CpG island. A) R-loop forming potential in the *HOTAIR-N-HOXC11* locus is illustrated using the UCSC Genome Browser. RFLS sequences are the predicted R-loop forming motifs in the *HOTAIR* locus. Each track is labeled on the left. DRIP-SEQ tracks illustrate the peaks of ChIP-SEQ reads generated using an R-loop-specific antibody in various cell lines. **B)** A possible mechanism for R-loop-mediated activation of HOTAIR expression is illustrated. The purple lines indicate DNA. The yellow line indicates RNA. The DNA-RNA hybridized bubble region indicates R-loop. The short brown bars indicate methylated cytosines. The Pol II oval indicates RNA polymerase II. The red crosses indicate transcriptional silencing.

instance, *HOTAIR* acts as a competitive endogenous RNA (ceRNA) to sequester miR-331-3p through a complementary target site in the last exon of *HOTAIR*, thereby abrogating miR-331-3p-mediated repression of the oncogene HER2 [116]. In gall bladder cancer cells, *HOTAIR*'s oncogenic activity requires the binding and neutralizing of miR-130a via a target site in its last exon [117]. In renal interstitial fibroblasts, *HOTAIR* activates the Notch1/Jagged1 signaling pathway by acting as a ceRNA for miR-124 [118]. In osteosarcoma, *HOTAIR* sequesters miR-217 via direct binding, releasing the oncogene ZEB1 from miR-217-mediated inhibition [119]. In nasopharyngeal carcinoma cells, *HOTAIR* binds to miR-101, thus abrogating the inhibition of COX-2 by this miRNA [75]. In general, *HOTAIR* acts as a ceRNA to sequester tumor suppressive miRNAs, thereby increasing the pool of available and active oncogenes in a variety of cancers. However, one caveat for this paradigm is that the amount of *HOTAIR* in a given tumor may be insufficient to sequester miRNAs because *HOTAIR*, like many lncRNAs, is estimated to be expressed much less abundantly than most protein coding genes and miRNAs.

5. Challenges and future directions

Despite recent advances in our understanding of *HOTAIR* function, significant obstacles remain on the path to realizing the diagnostic and therapeutic potential of *HOTAIR* in cancer. One challenge is the lack of a high-resolution map of *HOTAIR*'s interactions with its protein partners. NMR and crystallography experiments to characterize the complexes formed by *HOTAIR* and its binding proteins are urgently needed in order to design compounds that can specifically disrupt interactions between *HOTAIR* and its oncogenic partners. This need is highlighted by the fact that PRC2 physically interacts with thousands of lncRNAs and these interactions tightly regulate its function [119,120]. In addition, the discovery of RNA G4s in *HOTAIR* presents an appealing avenue to explore the precise disruption of *HOTAIR*'s interaction with PRC2 in cancer because tools and reagents are readily available to characterize and manipulate RNA G4s [11–13].

It is also anticipated that *HOTAIR* interacts with and regulates a host of proteins in cancer besides PRC2, LSD1, and E3 ubiquitin ligases, which have already been characterized. Therefore, the versatile functions of *HOTAIR* in cancer need to be explored through a thorough screening of *HOTAIR*-bound protein partners by tagging endogenous *HOTAIR* in cancer cells. The CRISPR-CAS9 gene editing technology is a promising tool for inserting an MS2 tag at a desirable site in the *HOTAIR* gene locus, which would enable affinity purification of MS2-tagged HOTAIR followed by mass spectrometry to identify novel protein partners of endogenous *HOTAIR* in various cancers [120]. Recent advances in CRISPR-CAS9 technology, especially recent success in genome-wide screening of therapeutically actionable lncRNAs in cancer cells, lay the foundation to dissect the role of *HOTAIR* via precise editing at single nucleotide resolution in cancer [121].

Recognition of *HOTAIR-N* as the major isoform in cancer undoubtedly provides new avenues for research. First, *HOTAIR-N* is transcribed from the 1st intron of *HOXC11* and forms a sense-antisense gene pair with *HOXC11*. Within the *HOTAIR-N-HOXC11* overlapping region there is a strong CpG island that has 160 CpG sites (Fig. 1) [122], which presents an ideal platform for investigating the dysregulation of a sense-antisense gene pair in the context of a CpG island in cancer. This overlapping configuration also implies *cis* actions of *HOTAIR* in cancer cells besides its established *trans* actions [5,99]. This notion is appealing because HOXC11 promotes breast cancer and the importance of PRC2 to *HOTAIR* functions has been challenged recently [123,124]. Potential *cis* regulation of the *HOTAIR-N-HOXC11* locus is plausible because of the tight correlation between *HOTAIR* and *HOXC11* expression in tumor tissues, such as those found in the TCGA lung adenocarcinoma cohort (Pearson: 0.73; Spearman: 0.92) and in cancer cell lines (unpublished observations) [102]. Furthermore, the discovery of DNA looping between *HOTAIR* and the distal *HOXC* enhancer gives rise to another potential *cis* action of *HOTAIR* in that transcriptional activation or repression codes in the *HOTAIR* locus may be transferred to its connected loci, thus regulating gene expression distally *in cis* [24,104]. In this case, chromatin conformation capture assays could be applied to discover the distal loci connected with the *HOTAIR* locus as well as potential reciprocal regulation of *HOTAIR* and its connected loci in cancer cells. A thorough understanding of *cis* regulation within the *HOTAIR-N-HOXC11* locus is essential because it could potentially lead to the ability to silence multiple tumor promoting genes with one strike. Moreover, the knowledge gained from the *HOTAIR-N-HOXC11* locus is particularly important because it will offer general insight into the global dysregulation of sense-antisense gene pairs that commonly occurs in cancer [125].

6. Summary

HOTAIR has emerged as a master regulator of cancer and a promising target for diagnostics and therapeutics. Materialization of *HOTAIR*'s clinical potential will require further investigation of the molecular mechanisms underlying dysregulation of its expression and function in cancer cells. Finally, knowledge acquired from investigations of *HOTAIR* will shed light on our understanding of other lncRNAs in cancer.

Conflicts of interest

The authors declare they have no conflict of interest related to this submission.

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