HHS Public Access

Author manuscript

Biochim Biophys Acta. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

Biochim Biophys Acta. 2015 August ; 1856(1): 151–164. doi:10.1016/j.bbcan.2015.07.001.

LncRNA HOTAIR: a master regulator of chromatin dynamics and cancer

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Abstract

Non-coding RNAs (ncRNAs) are emerging classes of regulatory RNA that play key roles in various cellular and physiological processes such as in gene regulation, chromatin dynamics, cell differentiation, development etc. NcRNAs are dysregulated in a variety of human disorders including cancers, neurological disorders, and immunological disorders. The mechanisms through which ncRNAs regulate various biological processes and human diseases still remain elusive. HOX antisense intergenic RNA (HOTAIR) is a recently discovered long non-coding RNA (lncRNA) that plays critical role in gene regulation and chromatin dynamics, appears to be misregulated in a variety of cancers. HOTAIR interacts with key epigenetic regulators such as histone methyltransferase PRC2 and histone demethylase LSD1 and regulates gene silencing. Here, we have reviewed recent advancements in understanding the functions and regulation of HOTAIR and its association with cancer and other diseases.

1. Introduction

Non-coding RNAs (ncRNAs) are emerging classes of transcripts that play major regulatory roles in various biological processes [1, 2]. Recent studies based on Encyclopedia of DNA Elements (ENCODE) project indicate that more than 80% of the human genome contains functional DNA elements [1, 3] that includes protein coding genes, non-protein coding regulatory DNA elements, binding sites for transcription factors, and ncRNAs [4–6]. While the existence of large numbers of non-coding transcripts (ncRNAs) are well recognized, their biological function and potential of being translated is controversial and is a major focus of current research. Initially, due to lack of obvious open-reading-frame (ORF) as well as lack of conservation in different organism, it was assumed that transcripts are non-coding. However, recent attempts to assess the genome-wide translation potential, suggest that majority of these transcripts are non-coding in nature, and there are subsets of ncRNAs that are translated, though the functional significance of the translated peptides are yet to be revealed [7, 8]. Genome wide ribosome profiling analysis revealed that there are much more widespread translational activities than anticipated indicating translation potential of some

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of the so-called non-coding transcripts [9–11]. NcRNAs plays crucial roles in maintaining genome packaging, chromatin dynamics and gene regulation. NcRNAs are classified into three major groups on the basis of their lengths: short ncRNAs (20–50 nt long: such as miRNAs, siRNAs, etc.), medium ncRNAs (50–200 nt long: such as snRNAs, snoRNAs, PROMPTs, TSSa-RNAs etc.), and long ncRNAs (> 200 nt long: such as HOTAIR, MALAT1, H19, XIST and others) [4, 5]. Though many ncRNAs are being discovered rapidly, their detailed cellular functions and mechanisms of action still remain elusive. HOTAIR (HOX antisense intergenic RNA) is an example of lncRNA that plays major roles in gene regulation, chromatin dynamics, and cancer [12–15]. Herein, we have reviewed recent literatures showing the cellular functions, mechanism of action of HOTAIR and its implications in cancer and other diseases.

2. LncRNA HOTAIR

HOTAIR is a 2.2 kb long lncRNA that is transcribed from antisense strand of HOXC gene cluster present in chromosome 12 (Figure 1) [12]. Like protein coding genes, HOTAIR is transcribed by RNA polymerase II, spliced, polyadenylated and 5'-capped [5, 16, 17]. HOTAIR was originally discovered based on tiling microarray analysis by Rinn *et. al*. in 2007 and they showed that HOTAIR is located at the HOXC locus in chromosome 12, flanked by HOXC12 and HOXC11 (Figure 1). Strand specific reverse transcriptase PCR analysis confirmed that HOTAIR is transcribed in an antisense manner with respect to HOXC genes [12]. HOTAIR transcript's half-life is approximately 4 h in *HeLa* cells [18].

Many ncRNAs such as microRNAs that are found in plants and animals are well conserved across various species. However, HOTAIR appears to be not so well conserved across the evolution [19, 20]. HOTAIR is well-conserved among primates (99.5% and 95%, sequence identity in chimp and macaque genomes, respectively) [12]. Structure based RNA homology search revealed that orthologs of HOTAIR, though not highly conserved, exists in other mammals such as mouse and rat (Figure 2) [20]. The murine HOTAIR has about 58% sequence similarity to human HOTAIR [19], while rat HOTAIR has approximately 50% sequence similarity [19, 21]. HOTAIR is not found in non-mammalian vertebrates [20]. Human HOTAIR is comprised of 6 exons (exon 1, exon 2, exon 3, exon 4, exon 5, and exon 6), while mouse and rat HOTAIR has 5 exons, where exon 2 (human analog) is missing [21]. The other exons (exons 1, 3, 4, 5 and 6) are fairly conserved (50–60 % homology) in both mouse and rat, in comparison to the human HOTAIR [21]. The exon 6 of rat and mouse has two potential domains: domain A (~235 bp long) and domain B (~239 long) (Figure 2) [21]. Domain B appears to have more conservation than domain A, in mouse and rats [19, 21]. The sequence of the exon 6 ortholog is significantly shorter in rat than in mouse and humans (Figure 2) [19, 21]. Though the biochemical function of HOTAIR is well-studied in humans [5, 14, 22–24] and mice [19, 21], little is known about its functionality in rat or other organisms [21].

3. Functions of HOTAIR and its mechanism of action

3.1 HOTAIR regulates chromatin dynamics and induces gene silencing via interaction of histone methylase (PRC2) and histone demethylase (LSD1)

Following the discovery of HOTAIR, Rinn *et. al*. investigated its potential cellular functions [12]. These studies demonstrated that siRNA-mediated knockdown of HOTAIR leads to transcriptional activation of HOXD locus genes present in chromosome 2, including HOXD8, HOXD9, HOXD10 and HOXD11 [12]. However, there was no significant effect on the transcription of HOXC cluster genes present in chromosome 12, where HOTAIR is actually coded [25]. HOXB locus was also not significantly affected by HOTAIR depletion [12]. These observations indicate that HOTAIR regulates gene expression *in trans* fashion and was the first lncRNA to be discovered to regulate gene expression *in trans* fashion (Figure 6) [12]. ChIP-chip analyses showed that HOTAIR knockdown results in a significant loss of H3K27-trimethylation marks at the HOXD locus. Notably, H3K27-methylation (H3K27-me3) is well recognized as the hallmark of gene silencing and is introduced by histone methyl-transferase EZH2 (Enhancer of Zeste homolog 2), which is a member of polycomb repressive complex 2 (PRC2) [5]. PRC2 is one of the two classes of polycomb-group proteins [26]. The core PRC2 complex comprises four components: EZH2, SUZ12 (suppressor of zeste 12 homolog), EED (embryonic ectoderm development) and RbAp46/48 (retinoblastoma associated protein 46/48) (also known as RBBP7/4, retinoblastoma binding protein 7/4) [4, 27]. Interestingly, along with the loss in H3K27-trimethyl marks, knockdown of HOTAIR also resulted in loss of occupancy of SUZ12 in the HOXD loci [25]. Occupancy levels of SUZ12 and H3K27-trimethyl marks were not affected at the silent HOXB locus, suggesting that HOTAIR is required to selectively target PRC2 complex to silence the transcription of HOXD locus [12]. Immunoprecipitation of SUZ12 from nuclear extracts of primary fibroblasts retrieved associated endogenous HOTAIR lncRNA [12]. In the reciprocal experiment, biotinylated-HOTAIR retrieved PRC2 components such as SUZ12 and EZH2. Collectively, these experiments demonstrated that HOTAIR interacts with PRC2 (containing H3K27 methylase EZH2 and other subunits) and guides it to the target HOXD locus, that results in H3K27-trimethylation and target gene/loci silencing (Figure 3) [12, 25].

HOTAIR was further shown to interact with histone demethylases LSD1 (lysine specific demethylase 1A, also called KDM1) [28]. LSD1 is a flavin-dependent monoamine oxidase, which demethylates lysines, specifically lysine 4 on histone 3 (H3K4) [28]. LSD1 is known to form a multi-protein complex with REST (RE1-Silencing Transcription factor) and CoREST that are critical players in gene silencing [29]. Indeed, based on immunoprecipitation analyses, Tsai *et. al* showed that HOTAIR interacts with REST and CoREST along with LSD1 [29]. Using HOTAIR deletion mutants, Tsai *et. al*. mapped the binding sites of PRC2 and LSD1 to different regions of HOTAIR lncRNA [29]. These studies demonstrate that HOTAIR lncRNA possess distinct binding domains for PRC2 and LSD1; PRC2 binds to the 5’-end of HOTAIR (1–300 nt) and LSD1 binds to the 3’-end of HOTAIR (1500 to 2146 nucleotides) [28]. HOTAIR acts as a modular scaffold and interacts with PRC2 and LSD1 complexes directly, recruits them to the target gene loci and represses

their transcription via H3K27-trimethylation (PRC2 activity) and H3K4-demethylation (LSD1 activity) (Figure 3) [28, 29].

In an independent study, Wu *et. al*. investigated binding interactions between PRC2 and HOTAIR. Specifically they investigated the interactions between PRC2-core catalytic heterotrimer (EZH2-EED-SUZ12, called as PRC2-3m) and HOTAIR. These studies identified a minimal HOTAIR binding motif, 89 nucleotides long that is responsible for PRC2-3m interaction [30]. Further, deletion analyses demonstrated that loss of nucleotides from this region results in significant loss of binding affinity of PRC2 to HOTAIR [30]. The binding affinity of the EZH2-EED-complex to HOTAIR is similar to the binding affinity of the PRC2-3m complex [30]. Nuclease mapping and foot-printing studies showed that this minimal binding element in HOTAIR lncRNA possesses a highly structured RNA domain consisting of secondary structure [30].

In contrast to above studies, Davidovich *et. al*. demonstrated that the binding affinity of PRC2 is not very specific to HOTAIR, rather, the binding affinity of PRC2 to any random RNA is comparable to that of HOTAIR. The PRC2 binding affinity appears to be dependent on size of the RNA rather than on the secondary structure [31]. Thus, promiscuous RNA binding phenomena demonstrated by PRC2 may serve as a checkpoint to prevent escape from silencing [31]. Furthermore, the binding affinity of PRC2 to any types of RNA is higher than the binding affinity of EED (component of PRC2) to the repressive-mark histone-tail peptides such as H3K27me3-, H3K9me3- and H1K26me3-, indicating high affinity of PRC2 complex towards any RNA, but not specifically towards HOTAIR [31]. Thus, the studies by Davidovich *et. al*. appears to be in contradiction with the studies by Wu *et. al*. and therefore, further studies are required for understanding the specific roles of HOTAIR in PRC2 recruitment to various genomic loci.

Recent studies also indicate that HOTAIR binds GA rich polypurine DNA motifs on the chromatin loci that regulate gene transcription (Figure 3) [32]. In order to understand if HOTAIR actively recruits PRC2 to its target genes or just acts as a molecule that gets transported along with PRC2 and does not affect the recruitment of PRC2, genome wide chromatin occupancy level of HOTAIR was examined in the absence and presence of EZH2 (component of PRC2), using ChIRP-qPCR (Chromatin Isolation by RNA Purification-qPCR). These analyses showed that the pattern of HOTAIR occupancy at HOTAIR target genes loci are preserved even in the absence of EZH2 suggesting that HOTAIR is an active recruiter of chromatin modifying complexes including EZH2 [32]. To understand the function of HOTAIR *in vivo*, Schorderet *et. al*. deleted the HOXC cluster whereby all HOXC genes and intergenic transcripts (e.g. HOTAIR) were deleted [19]. Importantly, deletion of the whole HOXC cluster leads to neonatal lethality [33, 34]. HOXC cluster deletion also results in skeletal abnormalities in HOXC null mice [35]. Studies have also shown that the HOXC genes are important for the overall body plan of mouse [35]. Deletion of mouse HOTAIR (mHOTAIR) produced no obvious phenotype in mice embryo at embryonic day 11.5, 12.5 and 13.5 [19]. There was no apparent deregulation of canonical HOTAIR target genes HOXD10 [19]. These observations indicate that evolution of HOTAIR has occurred faster than the neighboring HOXC genes and has acquired a

functional importance in humans that is not easily revealed in mice [19]. *In vivo* role of HOTAIR still requires specific HOTAIR-deficient mouse model [19].

3.2 BRCA1 competes with HOTAIR for binding to EZH2

Breast cancer susceptibility gene 1 (BRCA1) is a critical player in DNA damage response and in maintaining genomic integrity [36]. A recent study showed that BRCA1 interacts with EZH2 [36]. Specifically, immunoprecipitation of EZH2 (using myc-EZH2) pulls down BRCA1. GST-BRCA1 pull down analysis further confirmed that BRCA1 specifically interact with EZH2 [36]. BRCA1 interacts with 341–559 amino acid domain of EZH2 and this BRCA1 interacting region in EZH2 is also shown to be involved in recognition of ncRNA. Thus, the region of EZH2 that interacts with BRCA1, overlaps with the ncRNA binding domain 1 (ncRBD1, amino acids 342–370) of EZH2 [36]. Since ncRBD1 of EZH2 is necessary for the interaction with HOTAIR and overlaps with BRCA1 recognition site, BRCA1 mediated inhibition of EZH2 and HOTAIR interaction was investigated. Interestingly, knockdown of BRCA1 resulted in increased interaction between EZH2 and HOTAIR, indicating potential competition between HOTAIR and BRCA1 for the overlapping binding site on EZH2 [36]. Furthermore, overexpression of BRCA1 interfered with HOTAIR target gene (such as HOXA9) silencing via inhibition of EZH2 at the target gene (HOXA9) promoter [36]. These findings indicate that BRCA1 and HOTAIR bind to PRC2 complex member EZH2 and thus, HOTAIR may regulate expression of various genes by competing with other proteins such as BRCA1 (Figure 3) [36].

3.3 Phosphorylation of EZH2 may regulate its interaction with HOTAIR

Post-translational modification plays crucial roles in protein-protein and protein-nucleic acids interaction. In a recent study, Kaneko *et. al*. investigated the interaction of EZH2 with HOTAIR and its potential regulation by post-translation modification. These studies showed that the interaction of HOTAIR with EZH2 is regulated by site-specific phosphorylation in EZH2. The binding affinity of HOTAIR to EZH2 is increased when EZH2 is phosphorylated at threonine-345 (T345), in comparison to non-phosphorylated EZH2 (Figure 3) [37]. EZH2 phosphorylation is carried out by cyclin dependent kinase CDK1 and is cell cycle dependent (Figure 3) [37]. The levels of phosphorylated EZH2 is low at G1 and that is increased at G2/M phase [37]. To further investigate if PRC2 interaction with HOTAIR is mediated through EZH2 and, in particular, if this interaction is regulated by EZH2 phosphorylation, the characteristic negative charge of a phosphorylated threonine (Thr) residue within EZH2 protein was mimicked by substitution with a negatively charged aspartic acid residue. This phosphomimetic mutation at residue Thr-345 resulted in increased HOTAIR binding to EZH2, in comparison to the non-phosphorylated EZH2. Similar to phosphorylation of Thr 345 in murine EZH2, in humans EZH2 was found to be phosphorylated at Thr 350 [37]. The human EZH2 is phosphorylated by CDK1 and CDK2 (Figure 3) [37]. However the functional significance of the T350 phosphorylation in human EZH2 still remains elusive [37]. Taken together, these observations demonstrate that the interaction of HOTAIR with EZH2 and its potential cellular functions is regulated by post translational modifications such as phosphorylation at threonine 345 in EZH2 [37].

3.4 HOTAIR functionally interacts with E3 ubiquitin ligases

A recent study reported that HOTAIR acts as a platform for protein ubiquitination [13]. HOTAIR interacts with E3 ubiquitin ligases (Dzip3 and Mex3b) bearing RNA-binding domains as well as with the respective ubiquitination substrates, such as Ataxin-1 and Snurportin-1 (Figure 6) [13]. Notably, Ataxin-1 is a critical player in neuronal development and its mutation is linked with various neurological disorders including spinocerebellar ataxia type 1 [38]. Snurportins are family of proteins involved in nuclear transport, snRNA assembly and mRNA splicing [39]. HOTAIR facilitates ubiquitination of Ataxin-1 by Dzip3 (DAZ Interacting Zinc Finger Protein 3), both of which are cytoplasmic in localization. HOTAIR also aids in the ubiquitination of Snurportin-1 by Mex3b (mex-3 RNA binding family member B), both Snurportin-1 and Mex3b are nuclear as well as cytoplasmic in localization. Thus, HOTAIR promotes degradation of Snurportin-1 and Ataxin-1 via ubiquitination, not only in the nucleus but also in the cytoplasm [13], indicating potential involvement of HOTAIR facilitated ubiquitination and protein degradation in both cytoplasm and nucleus [13]. Furthermore, Mex3b associates with other signaling proteins such as SMAD4 (SMAD family member 4) and NUDT3 (nucleoside diphosphate linked moiety X-type motif 3, a homoeostasis checkpoint protein) [40, 41]. The interaction of HOTAIR with Mex3b further suggests the potential involvement of HOTAIR in other cell signaling processes [13]. Notably, this study showed that HuR (human antigen R, a key player in mRNA stability and gene expression, [42]) binds to a segment of HOTAIR that also interacts with Dzip3, Ataxin-1 and Snurportin-1. HuR is shown to downregulate HOTAIR. It has been suggested that at low levels of Ataxin-1 and Snurportin-1, HuR might bind to HOTAIR, reducing its stability and inhibiting the processes of ubiquitination and proteolysis of Ataxin-1 and Snurportin-1. However, when the levels of the Ataxin-1, Snurportin-1 or Dzip3 are high, their binding to HOTAIR prevents the interaction of HuR with HOTAIR, leading to the increased stability of HOTAIR and thus facilitating the ubiquitination and degradation of Ataxin-1 and Snurportin-1 [13]. Furthermore, the HOTAIR expression levels were found to be highly upregulated in senescent cells, causing rapid decay of targets Ataxin-1 and Snurportin-1, and preventing premature senescence [13]. These studies uncovered a novel role of HOTAIR, as a platform for protein ubiquitination, where HOTAIR helps in assembling both E3-ubiquiting ligases and their respective substrates. [13]

In a more recent study, HOTAIR expression has been linked to Plk1 (polo-like kinase 1, a Serine/threonine-protein kinase) -dependent ubiquitination of SUZ12 and ZNF198 [43]. Cells with Hepatitis B virus (HBV) exhibit increased expression of Plk1 kinase and reduced levels of SUZ12 and ZNF198. SUZ12 is a member of PRC2-compex and ZNF198 stabilizes the transcription repressive complex composed of LSD1, Co-REST, and HDAC1. HOTAIR is the common interactive member of both PRC2 and LSD1-complexes. In this study, authors show that Plk1 induces proteasomal degradation of SUZ12 and ZNF198 and Plk1-dependent ubiquitination of SUZ12 and ZNF198 is increased upon HOTAIR expression. The downregulation of SUZ12 and ZNF198 in cells with HVB contributes to global changes in histone modifications and altered epigenetic programming. Notably, liver tumors from X/c-myc bi-transgenic mice show decrease in SUZ12 and ZNF198 expression and increase

in PLK1 and HOTAIR expression [43], Thus, this analysis further links the potential involvement of HOTAIR expression with ubiquitination and proteasomal degradation.

3.5 HOTAIR acts as a competitive endogenous RNA (ceRNA)

A recent report identified that HOTAIR regulates the levels of micro RNAs via direct recognition followed by the target degradation. For example, HOTAIR possesses a binding site for miRNA-130a and this binding site is important for the regulation of miRNA-130a by HOTAIR (Figure 6). Biochemical analysis shows that HOTAIR-knockdown induces the miRNA-130a levels, while ectopic expression of HOTAIR reduces the miRNA-130a level, in gallbladder cancer cells [44]. On the other hand, miRNA-130a inhibitor upregulated HOTAIR level while miRNA-130a mimic repressed HOTAIR level, suggesting HOTAIR and miRNA-130a may form a reciprocal repression feedback loop [44]. A negative correlation between HOTAIR and miRNA-130a was also observed in gallbladder cancer tissues, providing evidence to such a feedback loop. Furthermore, based on RNA-IP (RNA immunoprecipitation) experiments it was shown that HOTAIR and miRNA-130a are bound to the same RISC (RNA-induced silencing complex) complex [44].

In an independent study, it was shown that HOTAIR acts as a competitive endogenous RNA (ceRNA) for miR-331-3p and miR-124 and regulates their cellular levels [45]. Briefly, miR-331-3p interacts with human epithelial growth factor receptor 2 (HER2) and down-regulates HER2 levels. Notably, in carcinomas, HER2 acts as an oncogene, encoding a protein to trigger the activation of cell signaling networks, impacting on various malignant cell functions such as proliferation, motility, angiogenesis and apoptosis [46–48]. Interestingly, it was shown that miR-331-3p is an interacting partner for HOTAIR. Thus, competitive binding of HOTAIR to miR-331-3p would alleviate the miR-331-3p–mediated suppression of HER2 expression. Indeed, HOTAIR knockdown was shown to decrease the expression level of HER2 gastric cancer cells such as BGC-823, while its overexpression restored elevated HER2 levels [45, 49]. Furthermore, RT-qPCR analyses showed that miR-331-3p/miR-124 expression was inversely correlated with HOTAIR expression in advanced gastric cancer [45].These observations demonstrate the importance of HOTAIR in the tumorigenesis-regulating network by regulating the expression of HER2, a target of miR-331-3p, through competition for miR-331-3p (Figure 6). Another recent study demonstrated that HOTAIR also negatively regulates miR-218 expression [50]. Ectopic expression of HOTAIR reduces miR-218 expression while its depletion promotes miR-218 expression [50]. HOTAIR has been shown to competitively bind to miR-193a in AML cells and functions as a ceRNA to modulate c-KIT (proto-oncogene) expression [51].

4. Transcriptional regulation of HOTAIR

4.1 HOTAIR is transcriptionally regulated by estradiol

HOTAIR, being a critical player in gene silencing, its expression must be tightly regulated in cell. HOTAIR promoter contains variety of transcription factor binding sites that includes multiple estrogen response elements (EREs), Sp1 binding sites, hypoxia response elements (HREs), CpG-islands, AP1 binding sites etc., indicating its potential regulation by diverse factors. (Figure 4) [5, 16, 17]. In a recent study, we observed that HOTAIR expression is

transcriptionally activated by estradiol in estrogen receptor (ER) positive breast cancer cells [17]. Luciferase based reporter assay demonstrates that ERE2 and ERE3 that are located at −1486 bp and −1721 bp upstream from the transcription start site (+1) (Figure 4) of HOTAIR are functional and involved in estradiol-mediated HOTAIR gene activation [17]. ERs bind to HOTAIR promoter EREs in an estradiol-dependent manner [17]. Knockdown of either ERα or ERβ, abolished the estradiol-induced HOTAIR expression [17], indicating critical roles of ERs in estradiol-mediated regulation of HOTAIR expression.

Estradiol-mediated gene activation may follow diverse mechanisms. In a typical genomic mechanism, upon binding to estradiol ERs get activated and bind to EREs present in the target gene promoters. Along with ERs, various ER-coregulators, histone acetyl-transferases and methyl-transferases, may associate with the estradiol-regulated gene promoters, modify and remodel chromatins and ultimately induce target gene expression [52, 53]. Studies from our laboratory and others have demonstrated that mixed lineage leukemia (MLL) family of histone methyl-transferases (MLLs) that are critical players in gene activation [16, 54–61]; [62, 63] act as ER-coregulators and regulate various estradiol-dependent activation of HOTAIR [17]. Specifically, MLL1 and MLL3 bind to the HOTAIR promoter in the presence of estradiol (Figure 5). Additionally levels of other ER-coregulators such histone acetyl-transferases CBP/p300 and also the levels of histone H3 lysine-4 trimethylation and histone acetylation are enriched at the HOTAIR promoter, in an estradiol dependent manner (Figure 5) [17].

Gene expression may be regulated at various levels. For example, in the absence of any external stimuli (such as estradiol), gene expression is maintained in the basal expression state. This may be mediated via involvement of various negative coregulators that binds to the gene promoter allowing no or basal level of transcription. In the presence of a stimuli, activators, co-activators, histone acetyltransferases, methyltransferases, etc. are recruited to the gene promoters, resulting in histone modification, chromatin remodeling and transcription activation. In fact, our studies demonstrated that N-CoR (Nuclear receptor Co-Repressor), a negative coregulator, is bound to both HOTAIR promoter in the absence of estradiol [16, 17] and its binding was decreased upon treatment with estradiol while the binding of ERs, MLLs and CBP/p300 were increased [16, 17, 64]. These observations suggested that HOTAIR transcription is originally maintained at the basal state by co-repressor N-CoR and the repression was relieved in the presence of estradiol which is mediated via binding of ERs and various ER-coactivators, upon exposure to estradiol [16, 17, 65–67]. Notably, HOTAIR is an antisense-transcript and therefore, our studies also demonstrated that similar to protein-coding gene transcription, estradiol-induced transcription of antisense transcript HOTAIR is coordinated via ERs and ER coregulators.

An independent study showed that HOTAIR is transcriptionally regulated by c-Myc, an oncoprotein and a transcription factor that plays critical role in tumorigenesis [68]. C-Myc induces HOTAIR expression through direct interaction with the E-box (c-Myc target response element) in the HOTAIR promoter region [68]. Ectopic expression of c-Myc increased HOTAIR expression and its promoter activity, while knockdown of c-Myc reduced HOTAIR expression and its promoter activity [68]. Nucleotide mutant in the E-box element in the promoter region abrogated c-Myc-dependent HOTAIR promoter activation

[68]. A positive correlation between expression levels of c-Myc and HOTAIR mRNA were also observed in gallbladder cancer tissues [68]. These observations suggested that c-Myc is an oncoprotein and its deregulation leads to gallbladder cancer potentially via misregulation of HOTAIR [68].

4.2 HOTAIR expression is regulated by microRNA

MicroRNAs (miRNAs) are critical modulators of gene expression and are involved in regulation of various biological processes including cell proliferation, cell differentiation and development [69–77]. Misregulation of miRNAs is closely associated with various diseases including cancer [78–80]. In a recent study, Chiyomaru *et. al*. demonstrated that miR-141 binds to HOTAIR in a sequence specific manner and suppresses HOTAIR expression and hence reduces cell proliferation and tumor invasion [81]. Suppression of HOTAIR levels by miR-141 is correlated with alteration of HOTAIR function. MiR-141 suppression of HOTAIR expression was found to be Argonaute2 (Ago2) dependent [81]. Immunoprecipitation studies demonstrated that HOTAIR interacts with miR-141 in the Ago2-complex and is cleaved by Ago2 in the presence of miR-141 [81]. The expression of miR-141 is inversely correlated with tumorigenicity and cancer invasiveness [81]. MiR-141 suppresses malignancy in cancer cells whereas HOTAIR has been demonstrated to promote malignancy [81]. Overall these studies demonstrate that HOTAIR is critically regulated via miR-141 and over expression of HOTAIR in cancer might be correlated with lowered expression of miR-141. In another study, Niinuma *et. al*. observed that the levels of miR-196a and HOTAIR are highly upregulated in high-risk gastrointestinal stromal tumors (GISTs), suggesting that HOTAIR and miR-196a both act in synergy to promote GIST cell invasion and malignancy [82].

4.3 HOTAIR expression is disrupted upon exposure to estrogenic endocrine disruptors

Endocrine disruptors are exogenous substances that alter the normal functioning of endocrine system and causes adverse health effects in humans [67, 83, 84]. Endocrine disruptors interact with hormone receptors even at very low concentrations and interfere with hormone signaling affecting various hormonally regulated processes including reproduction and development [67, 85, 86]. Endocrine disruptors are also capable of inducing aberrant and altered gene expression [85, 86]. Exposure to estrogenic endocrine disruptors such as bisphenol-A (BPA) and synthetic estrogens such as diethylstilbestrol (DES) alters uterine HOX gene expression and induces developmental changes in the female reproductive tract [87–89]. Both DES and BPA are agonists of estradiol that bind and activate ERs via interaction with their multiple phenolic moieties leading to alteration in ER target gene expression [16, 45, 60, 67, 90, 91].

In a recent study, we investigated the effect of estrogenic endocrine disruptors BPA and DES on the expression levels of HOTAIR both *in vitro* and *in vivo* (Figure 5). Our studies showed that HOTAIR expression is induced significantly upon exposure to BPA and DES *in vitro*, in cultured breast cancer cells MCF7 [16]. The expression levels of HOTAIR were also significantly augmented *in vivo*, in the mammary glands of ovariectomized Sprague dawley female rats that were exposed to acute levels of estradiol, BPA and DES [16, 17]. Biochemical studies demonstrated that HOTAIR promoter EREs are induced upon exposure

to BPA and DES (luciferase assay). ERs (ERα and ERβ) and ER-coregulators such as the MLL-family of histone methylases (MLL1 and MLL3), CBP and p300 were bound to the HOTAIR promoter upon exposure to BPA and DES (Figure 5). BPA and DES treatment altered the epigenetic marks such as histone H3K4-trimethylation, histone acetylation levels at the HOTAIR promoters and that resulted in HOTAIR gene activation [16]. Thus, exposure to BPA and DES alters the epigenetic programming at the HOTAIR promoter, which results in turning on HOTAIR gene expression, even in the absence of estradiol [16, 17].

Studies from our lab also demonstrated that HOTAIR interacting histone methyltransferase, EZH2, that specifically methylates histone 3 lysine 27 (H3K27), is also transcriptionally induced by estradiol, BPA and DES in cultured breast cancer cells and in the mammary glands of ovariectomized rats. Like HOTAIR, EZH2 promoter contains multiple functional estrogen-response elements where ERs and ER coregulators (such as MLL2, MLL3, CBP and p300) were enriched in an estradiol, BPA and DES dependent manner [60]. H3K4-trimethylation and histone acetylation levels were also increased at the EZH2 promoter in the presence of BPA and DES, resulting in EZH2 gene activation [16, 17]. As HOTAIR is a critical player in gene regulation and is often upregulated in a variety of cancers including breast cancer, we hypothesize that exposure of estrogenic endocrine disruptors such as BPA, DES and many others that are present in our environment, food products etc., may upregulate genes associated with cancer and contribute towards initiation and progression of cancer.

5. HOTAIR and cancer

Various reports have demonstrated that HOTAIR is a key regulator of chromatin dynamics and is misregulated in various carcinomas such as urothelial carcinoma [92], pancreatic tumors [93], hepatocellular carcinoma [94–97], colorectal carcinomas [98, 99], ovarian cancer tissues [100, 101], sarcomas [102], esophageal squamous cell carcinoma (ESCC) [103–106], renal carcinomas [20], nasopharyngeal carcinoma [107], gastrointestinal stromal tumors [82], Laryngeal squamous cell cancer [108, 109], gall bladder cancers [68], nonfunctional pituitary adenoma [110], prostate cancer [111], cervical tumors [109, 112, 113], melanomas [114], gastric cancers [45, 115, 116], Ta/T1 bladder cancer [72] and endometrial tumors [44, 117], (Table 1). Details are summarized below.

5.1 Breast carcinomas

Multiple studies demonstrate that the expression of HOTAIR is significantly upregulated in breast tumors [118–120]. HOTAIR expression level in primary tumors is correlated with metastasis and death potentials [118]. HOTAIR overexpression augments cancer invasiveness and metastases and its depletion inhibits cancer invasiveness. Overexpression of HOTAIR induces genome-wide re-targeting of PRC2-complex resulting in altered gene expression profiles, and that may contribute to tumor initiation, progression and metastasis [118]. In a separate study, Lu *et. al*. showed that the levels of HOTAIR expression varied widely in the primary breast tumors, however, there were no significant associations between HOTAIR expression and clinical or pathological characteristics such as overall or disease-free survival [120]. In multivariate analyses, patients with higher HOTAIR

expression had better survival potential than those with low expression [120]. They also showed that intergenic DNA methylation is positively correlated with HOTAIR expression levels and unfavorable outcomes including late disease stage, advanced tumor grade, large tumor size and negative status of estradiol and progesterone receptors [120].

Recent studies from our laboratory showed that HOTAIR expression is critical for the survival and proliferation of breast cancer cells (MCF7). In order to study the effect of knockdown of HOTAIR and analyze its impact on cell growth and viability, we developed a synthetic oligonucleotide DNA that is complementary to HOTAIR transcript and termed it as small interfering sense (siSENSE) oligonucleotide [17]. The normal phosphodiester bonds of the HOTAIR siSENSE DNA molecule were replaced with phosphorothioate linkage to minimize the nuclease digestion and enhance its *in vivo* stability. Our studies demonstrated that siSENSE-mediated knockdown of HOTAIR suppressed the growth of MCF7 and eventually induced apoptotic cell death. SiSENSE-mediated HOTAIR knockdown upregulated the expression levels of various genes such as cyclins, HOXD10, PCDH10, BCL2 and BID (BH3 interacting domain death agonist) [17]. HOXD10 plays a critical role in cell differentiation and morphogenesis during development. Expression of HOXD10 is downregulated in cancer tissues and is a potential tumor suppressor [121]. PCDH10 is broadly expressed in all normal adult and fetal tissues including the epithelia, though at different levels. Transcriptional silencing of PCDH10 and its promoter methylation are frequently detected in multiple carcinomas [122]. Ectopic expression of PCDH10 strongly suppresses tumor cell growth, migration, invasion and colony formation, suggesting that PCDH10 is a tumor suppressor [122]. HOTAIR appears to be negative regulator of these tumor suppressor genes and therefore HOTAIR overexpression may contribute to tumorigenesis via inhibition of multiple tumor suppressors.

Studies by Sorensen *et. al*. show that HOTAIR expression has a bimodal distribution in primary breast cancer and patients with metastatic breast cancer possess higher levels of HOTAIR expression [119]. Patients with low HOTAIR expression had better survival than those with high HOTAIR expression. This study also reports that ER-positive patients have higher HOTAIR levels and HOTAIR might be a potential prognostic marker in ER-positive breast cancer [119]. Another study shows that expression of HOTAIR and EZH2 are highly correlated, with strong positive HOTAIR expression, ER positivity and PR positivity [123]. On the contrary, subset of cases that showed strong EZH2 expression also correlated with an increased proliferation rate, ER- and PR-negativity [123]. HOTAIR and EZH2 had increased expression in the metastatic carcinomas [123]. However, co-expression of HOTAIR and EZH2 trended with the worst outcome [123]. A recent study reported that a secreted phosphoglycoprotein, osteopontin (OPN) that increases cell migration via EGFR (epidermal growth factor receptor) and MET (MET proto-oncogene, receptor tyrosine kinase) and is associated with tumor growth and metastasis, transcriptionally induces HOTAIR expression via upregulating the PI3K/AKT pathway (Figure 6) [124]. OPN decreases interferon regulatory factor-1 (IRF1) expression and further elevates the level of HOTAIR [125]. IRF1 binds to the HOTAIR promoter and regulates HOTAIR expression [125]. A recent report demonstrated that calycosin and genistein (Phytoestrogens of isoflavone class) inhibited proliferation and induced apoptosis in breast cancer cells (MCF7). Treatment of calycosin or

genistein also decreased phosphorylation of Akt, and also the expression of its downstream target, HOTAIR [126]. Mechanistic studies show that HOTAIR suppress a miRNA (miR-568) and in turn augments the NFAT5 (Nuclear factor of activated T cells 5) expression in metastatic breast cancers [127]. Another study showed that downregulation of miR-7 in breast cancer stem cells may be indirectly attributed to HOTAIR by modulating the expression of HOXD10 that promotes the expression of miR-7. These findings demonstrate that miR-7 is a tumor suppressor and its overexpression might serve as a good strategy for treating highly invasive breast cancer [128]. Alves *et. al*. evaluated the roles played by HOTAIR in epithelial-to-mesenchymal transition (EMT) and also in the maintenance of cancer stem cells (CSCs. Their study demonstrated that treatment with TGFβ1 (Transforming growth factor β1) induces HOTAIR levels and triggers EMT [129]. However, depletion of HOTAIR did not trigger TGFβ1 stimulated EMT program and also alleviated the colony-forming capacity of colon and breast cancer cells. HOTAIR acts as a key regulator of genes involved in the EMT [129]. Various studies also demonstrate that HOTAIR regulates various genes associated with cell cycle progression, cellular structural integrity, cell-cell signaling and development. These HOTAIR target genes include JAM2, PCDH10, PCDHB5 ABL2, SNAIL (snail family zinc finger 1), laminins HOXD10, PRG1 (P53-Responsive Gene 1) etc. [93, 94, 104, 108, 118, 129, 130].

5.2 Lung carcinomas

Multiple studies demonstrate that HOTAIR overexpression is associated with non-small cell lung carcinomas (NSCLC) and small cell lung cancers (SCLC). Patients with high HOTAIR expression levels, possessed higher lymphatic invasion and had significantly shorter survival times, in case of both SCLCs and NSCLCs [131–135]. HOTAIR overexpression increases cell migration and invasive ability [131, 133] and HOTAIR knockdown affects cell growth, impedes cell migration and ultimately induces apoptosis in NSCLCs and SCLCs [131, 133]. *In vivo* studies in nude mice also showed that HOTAIR knockdown decreases the number of metastatic nodules [131]. Suppression of HOTAIR upregulates the levels of HOXA5 and downregulates MMP2 (Matrix metallopeptidase 2) and MMP9 (Matrix metallopeptidase 9) that are critical players of tumor invasion and metastasis [131, 133].

Tumor microenvironment is enriched with interstitial extracellular matrix, such as type I collagen (Col-1) [135] . Col-1 up-regulates oncogenic miRNAs, such as miR-21 and promotes tumor progression [135]. Zhuang *et. al*. demonstrated that the levels of HOTAIR and col-1 were significantly higher in NSCLC tissues [135]. Col-1 supplementation induces the expression levels of HOTAIR [135]. Inhibition of integrin α2β1, a cell surface receptor for Col-1 results in reduced levels of HOTAIR, indicating potential regulation of HOTAIR by Col-1 [135]. Furthermore, HOTAIR promoter analysis identified four potential Myc binding sites. These findings suggest that Myc mediates activation of the HOTAIR gene by Col-1, since the levels of miR-17-92 cluster (another transcriptional target of Myc in cancer cells) were found to be significantly upregulated by Col-1 [135]. Cigarette smoke extract (CSE) exposure to human bronchial epithelial (HBE) cells induced IL-6 (Interleukin 6), a pro-inflammatory cytokine that activates STAT3 (Signal transducer and activator of transcription 3), a transcription activator. In turn, STAT3 interacts with the HOTAIR promoter leading to increased expression levels of HOTAIR (Figure 6) [48]. This study

suggests a link between inflammation, EMT, CSE and HOTAIR [48]. HOTAIR knockdown also altered the expression levels of various genes that include cell adhesion genes such as ASTN1 (astrotactin 1), PCDHα1 and 10 (protocadherin-α 1 and 10), and CLDN11 (Claudin-11), genes involved in mucin production such as MUC5AC (mucin 5AC, oligomeric mucus/gel-forming) and MUC4 [134].

An independent study investigated the role of HOTAIR in the resistance of lung adenocarcinomas (LAD) cells to cisplatin. They showed that HOTAIR expression was elevated in cisplatin-resistant A549/DDP cells as compared to normal A549 cells. HOTAIR knockdown resensitized the responses of A549/DDP cells to cisplatin both *in vitro* and *in vivo* [132]. In contrast, overexpression of HOTAIR decreased the sensitivity of A549 and SPC-A1 cells to cisplatin [132]. They showed that HOTAIR specific siRNA mediated chemosensitivity enhancement was associated with inhibition of cell proliferation, induction of G_0/G_1 cell-cycle arrest and apoptosis enhancement through regulation of $p21^{WAF1/CIP1}$ (p21) expression. These results imply that upregulation of HOTAIR leads to the cisplatin resistance of LAD cells, via regulation of p21 expression (Figure 6) [132].

Ovarian cancers—HOTAIR is known to plays a critical role in ovarian cancer [100, 101]. Several studies have already demonstrated that HOTAIR suppresses HOXD10 gene, mRNA that encodes a transcriptional repressor that inhibits the expression of cell migration and invasion-associated genes. However, a contradictory study, examined whether HOTAIR is able to regulate the expression of HOXD10 in epithelial ovarian cancer cell lines. HOTAIR depletion led to no significant upregulation of the HOXD10 protein and mRNA in the cells [101]. Another study identified a putative p65-NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) binding site 906 bp upstream of the HOTAIR transcription start site (TSS) [90]. Treatment of (A2780) ovarian cancer cells with the NF-κB activator, TNF-α induced HOTAIR expression and NF-κB enrichment at the HOTAIR promoter occurred in the presence of TNF-α [90]. The results of this study support a role for HOTAIR as a positive regulator of the NF-κB [90].

Hepatocellular carcinoma—A study reported that HOTAIR knockdown increased the expression of tumor suppressor gene, RNA binding motif protein 38 (RBM38) that is a critical player of cell migration, invasion and malignancy [94]. Thus, HOTAIR was shown to contribute toward migration and invasion of HCC cells by inhibiting RBM38 (Figure 6) [94].

Colorectal carcinomas (CRC)—HOTAIR levels in blood of sporadic colorectal cancer (CRC) patients were 4-fold higher as compared to the healthy controls, suggesting that HOTAIR blood levels may serve as potential surrogate prognostic marker in sporadic CRC [98]. Another study showed that HOTAIR knockdown suppressed the TGF-β1 induced epithelial-to-mesenchymal transition (EMT) and reduced the colony-forming capacity of colon cancer cells [136]. Colon cancer stem cell subpopulation (CD133(+)/CD44(+)) possesses higher levels of HOTAIR as compared to the non-stem cell subpopulations, suggesting that HOTAIR aides in carcinogenesis via stemness acquisition [136].

Pancreatic carcinomas—*In vitro* HOTAIR knockdown studies in pancreatic cancer cells further corroborated that HOTAIR is associated with enhanced cell invasion, cell proliferation, modulation of cell cycle progression, and induction of apoptosis [93]. In L3.6pL mouse xenograft models HOTAIR knockdown led to inhibition of pancreatic tumor growth, suggesting HOTAIR's anti-proliferative role in pancreatic cancer.

Esophageal squamous cell carcinoma (ESCC)—ESCC cells and tissues showed inverse correlation between HOTAIR overexpression and decreased WIF-1 (WNT inhibitory factor-1) expression, HOTAIR overexpression also promoted H3K27 tri-methylation in the promoter region of WIF-1 [104]. Notably, WIF-1 is a key inhibitor of the WNT/β-catenin signaling pathway and binds directly to extracellular WNT ligands, preventing their interaction with the receptors and leading to degradation of cytosolic β-catenin by the APC/Axin1 destruction complex (Figure 6) [104]. Epigenetic silencing of WIF-1 due to promoter hypermethylation is a frequent mechanism that causes aberrant activation of the WNT/β-catenin pathway in several human cancers, as well as in ESCC [104]. Generally, WIF-1 downregulation is a prominent characteristic of tumor progression [104]. Exogenously added recombinant human WIF-1 protein in ESCC cells, overexpressing HOTAIR, inhibited the migration and invasion ability, although, no correlations were found between WIF-1 protein level and prognosis of patients with ESCC [104].

Laryngeal squamous cell cancer (LSCC)—PTEN (Phosphatase and tensin homolog) is a critical player in laryngeal squamous cell carcinomas and PTEN promoter CpG island methylation is linked with LSCCs [108, 109]. Studies by Li *et. al*. showed that HOTAIR regulates the CpG methylation at the PTEN promoter suggesting potential roles of HOTAIR in PTEN associated carcinogenesis (Figure 6) [108]. Furthermore, another study identified HOTAIR in the LSCC patient's serum and that may serve as biomarker to screen LSCC [109].

Glioblastomas—Zhang *et. al*. carried out multivariate Cox regression analyses which showed that HOTAIR is an independent prognostic factor in glioblastoma patients [137]. HOTAIR knockdown in glioblastoma cell lines such as LN229 and U87 cells via RNAi not only demonstrated significant increase in the cells present in the G_0/G_1 phase but also altered the expression of various genes [137]. For example, the levels of cyclin D1, cyclin E, cyclin-dependent kinase CDK4, CDK2, and E2F1 were reduced and the levels of p21 and p16 expression were significantly increased [71, 137].

5.3 Other carcinomas

HOTAIR expression levels have been found to be significantly higher in variety of other cancers such as sarcomas, renal carcinomas, nasopharyngeal carcinoma (NPCs), gastrointestinal stromal tumors (GISTs), gall bladder cancers, nonfunctional pituitary adenoma, prostate cancer, cervical tumors, melanomas, gastric cancers, Ta/T1 bladder cancer, and endometrial tumors as compared to the adjacent normal tissues (Table 1) [20, 44, 49, 68, 70, 74, 82, 102, 107, 109–112, 117, 121, 122, 138, 139]. The levels of HOTAIR are higher in advanced tumors as compared to low grade tumors. Cancer patients with higher HOTAIR levels have poor prognosis for overall survival [5, 12, 13, 21, 22, 44, 68, 81, 82,

93, 95, 97–100, 102–106, 110, 111, 117, 119, 129, 130, 134, 135, 137–148]. HOTAIR has been identified as a novel biomarker and its expression levels can be used to analyze the degree of malignancy [5, 44, 68, 102, 110–112, 117]. HOTAIR augments the cancer invasiveness and metastasis [17, 24, 81, 94–96, 99, 104, 105, 108, 110, 111, 117–119, 130, 131, 137, 139, 142, 143, 149]. Poly r(C) Binding Protein (PCBP) 1 was suppressed by HOTAIR in gastric cancer *in vitro* and *in vivo* [126]. High expression of HOTAIR is associated with radio-resistance and downregulation of p21 in the primary cervical cancer cells [150]. HOTAIR expression is also found to be significantly high in leukemic cell lines and primary AML (Acute myeloid leukemia) blasts. AML patients with higher HOTAIR were predicted to have worse clinical outcome as compared to the patients with lower HOTAIR [51].

Recently, we analyzed the HOTAIR expression patterns in a variety of cancer using an online data base (http://www.cbioportal.org/public-portal/) which provides information about the mutations, amplifications and deletion in a variety of genes in various carcinomas based on RNA-Seq, microarray and other techniques [150, 151]. To generate the cross-cancer alteration summary, “Mutation and CNA (copy number alteration)” data types were analyzed for all cancer studies. These analyses revealed that HOTAIR copy number alterations (CNAs) and deletions are associated with a large number of carcinomas that include adrenocortical carcinoma, malignant peripheral nerve sheath tumors, adenoid cystic carcinoma etc. (Figure 7). These analyses, in addition with other individual studies, further support the argument that HOTAIR is a major player in various cancers, though further detailed studies are required to identify the role of HOTAIR in carcinogenesis.

6. Other Diseases

Beyond its association with cancer, HOTAIR is also associated with other disorders such as cardiovascular diseases and osteoarthritis (Table 1). Recently, it is reported that level of HOTAIR is decreased in the bicuspid aortic valve and in human aortic interstitial cells exposed to cyclic stretch [152]. HOTAIR knockdown results in higher expression of two genes, ALPL (alkaline phosphatase) and BMP2 (bone morphogenetic protein 2) that are required for calcification of aortic valve interstitial cells (AVICs) [152]. Microarray analyses showed that HOTAIR-knockdown results in activation of additional calcification related genes and pathways [112]. WNT/β-CATENIN signaling pathway plays a critical role in the aortic valve calcification. Treatment with WNT agonists increases ALPL and BMP2 expression and decrease in HOTAIR expression, showing that HOTAIR represses calcification-associated genes such as ALPL and BMP2. These data suggest that HOTAIR is mechano-responsive and is repressed by WNT/β-CATENIN signaling, suggesting involvement of HOTAIR in aortic valve calcification [112].

A recent study investigated the differences in expression levels of lncRNAs in osteoarthritic cartilages and in normal cartilage using microarray analyses. The analyses identified HOTAIR to be one of the lncRNA to be upregulated in osteoarthritic cartilages [153], suggesting that differential expression of HOTAIR may be associated with the pathogenesis of osteoarthritis [153]. Another study investigated the expression of HOTAIR in pre-eclampsia placentas and its effect on trophoblast cells [154]. Pre-eclampsia is a disorder

associated with pregnancy and is characterized by high blood pressure and a large amount of protein in the urine [154]. Pre-eclampsia increases the risk of poor outcomes for both the mother and the baby [154]. This study demonstrated that the levels of HOTAIR were significantly increased in severe preeclampsia groups as compared to normal pregnant placentas. The proliferation rate and invasive capacity of trophoblast cells (HTR-8/SVneo) was significantly decreased upon overexpression of HOTAIR while increased in HOTAIR knockdown conditions [154]. These observations suggest involvement of HOTAIR in the onset of pre-eclampsia by regulating proliferation, invasion and apoptosis of trophoblast cells. However more studies are required for understanding the molecular role of HOTAIR in pre-eclampsia [154].

A study showed that expression of HOTAIR, SHOX2 (short stature homeobox 2) and HOXC11 occurs in gluteofemoral adipose tissue [155], suggesting potential roles of HOTAIR in gluteofemoral adipose tissue biology [155]. Dermal papilla (DP) cells have been hypothesized to be the source of dermal derived signaling molecules involved in hair-follicle development and postnatal hair cycling [155]. HOTAIR is found to be aberrantly expressed in DP cells and play an important role in regulating WNT signaling [155]. These observations provide potential targets for identifying the hair-follicle induction mechanism of early-passage DP cells [155]. HOTAIR knockout mice studies have shown that there is a de-repression of numerous genes, resulting in homeotic transformation of the spine, loss of vertebral boundary specification during development and malformation of metacarpal-carpal bones [156]. HOTAIR appears to play pivotal roles in skeletal morphogenesis and embryonic patterning of the skeletal system *in vivo* [156].

7. Summary and conclusion

HOTAIR is a critical player in regulating chromatin dynamics. HOTAIR interacts with critical epigenetic regulators such histone methylase PRC2 and histone demethylase complex LSD1, recruits them to the target gene promoters, introduces histone H3K27-trimethylation and H3K4-demethylation and ultimately results in chromosome condensation and gene silencing [28]. HOTAIR regulates a variety of genes involved in cell cycle progression, cell migration, EMT, metastases, tumor progression etc. (Figure 6). HOTAIR overexpression is closely associated with a variety of cancers including breast cancer. Misregulation of HOTAIR potentially alters the epigenome and hence contributes to the alteration of cellular homeostasis and disease initiation and progression. HOTAIR expression may be regulated via diverse mechanisms such as via involvement of steroid hormones such as estradiol, miRNAs such as miR-141 (Figure 6). HOTAIR is a proto-oncogene and may be misregulated upon exposure to endocrine disrupting chemicals (EDCs) such as BPA and DES and that may contribute toward disease progression including carcinogenesis [16, 17].

The discovery of the involvement of HOTAIR in regulation of protein ubiquitination and protein degradation both in cytoplasm and nucleus opens up door for exploring the functions of HOTAIR beyond direct regulation of epigenome and chromosome dynamics [13]. The discovery of pivotal roles of HOTAIR in skeletal morphogenesis and embryonic patterning further signifies the importance of lncRNA HOTAIR in development. The association of

HOTAIR transcript levels to aortic valve calcification, osteoarthritis and pre-eclampsia etc. [13] suggest that HOTAIR may be associated with variety of physiological process and diseases beyond cancer. The detailed molecular mechanisms of diverse mode of HOTAIR regulation still remain elusive.

Acknowledgements

We thank all the Mandal lab members for helpful discussions. Research in Mandal laboratory is supported by grant from NIH (1R15 ES019129-01). We sincerely apologize to all the colleagues whose contributions are not cited here due to the limitation in the length of the article.

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Highlights

- **a.** HOTAIR is a long nom-coding RNA
- **b.** HOTAIR interacts with PRC2 and LSD1 and regulates gene silencing
- **c.** HOTAIR is overexpressed in variety of cancer
- **d.** We reviewed the functions of HOTAIR and its regulation
- **e.** We reviewed the associations of HOTAIR with cancer and other diseases

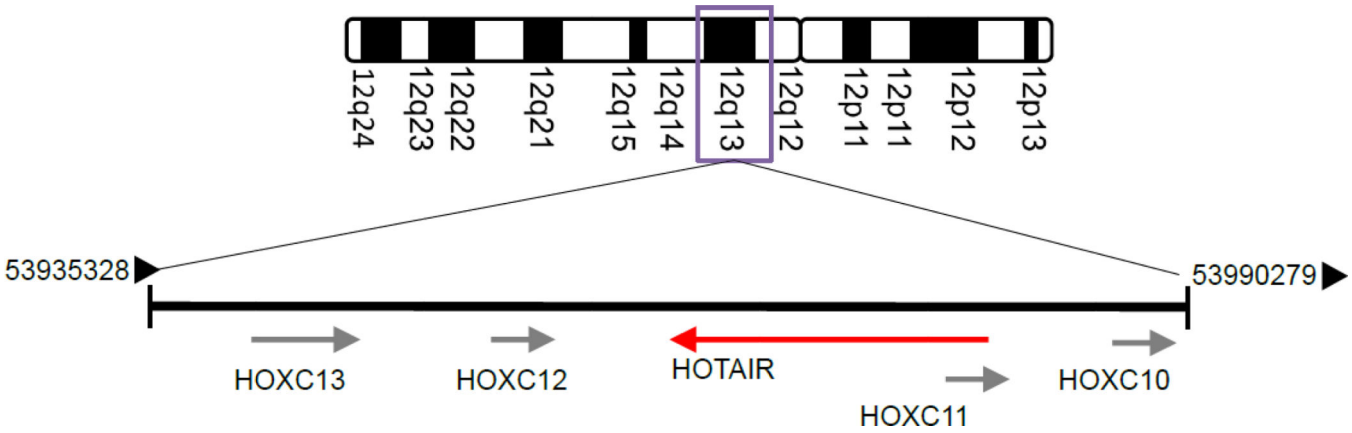

Figure 1.

Genomic location of HOTAIR. HOTAIR (2.2 kb long lncRNA) is transcribed from antisense strand of HOXC gene cluster from 12q13. HOTAIR is flanked by HOXC12 and HOXC11.

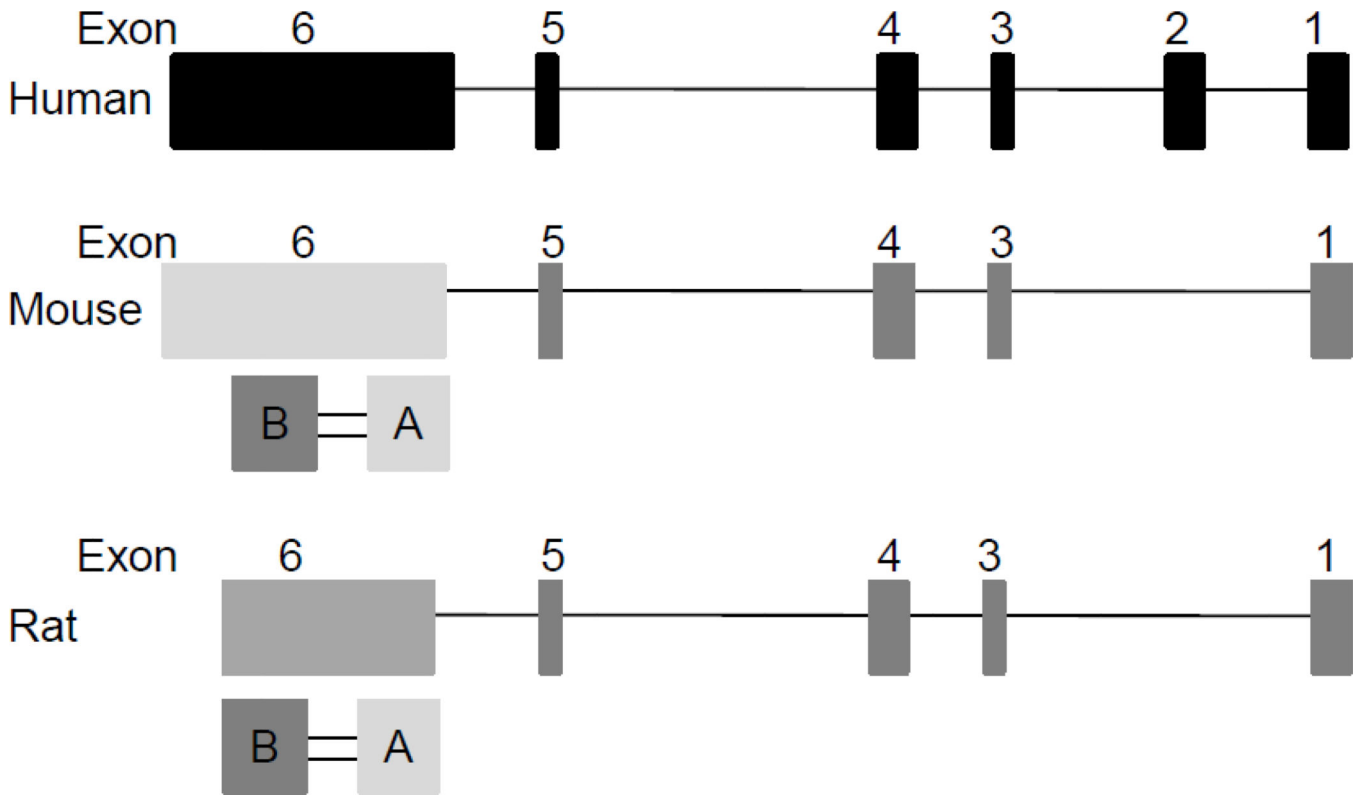

Figure 2.

Comparison of HOTAIR orthologs in rats, mouse and human. The murine HOTAIR has about 58% and rat HOTAIR has approximately 50% sequence similarity to human HOTAIR. Human HOTAIR is comprised of 6 exons (exon 1, exon 2, exon 3, exon 4, exon 5, and exon 6). Exon1, 3, 4, 5 and 6 are more conserved. Exon2 is absent in the mouse and rat HOTAIR. The exon 6 of rat and mouse has two hypothetical domains: domain A (~235 bp long) and domain B (~239 long). Domain B appears to have more conservation than domain A, in mouse and rats. The extent of sequence conservation is indicated by the darkness of the boxes, darker the color better conserved it is. The numbers on the top indicates the exon numbers

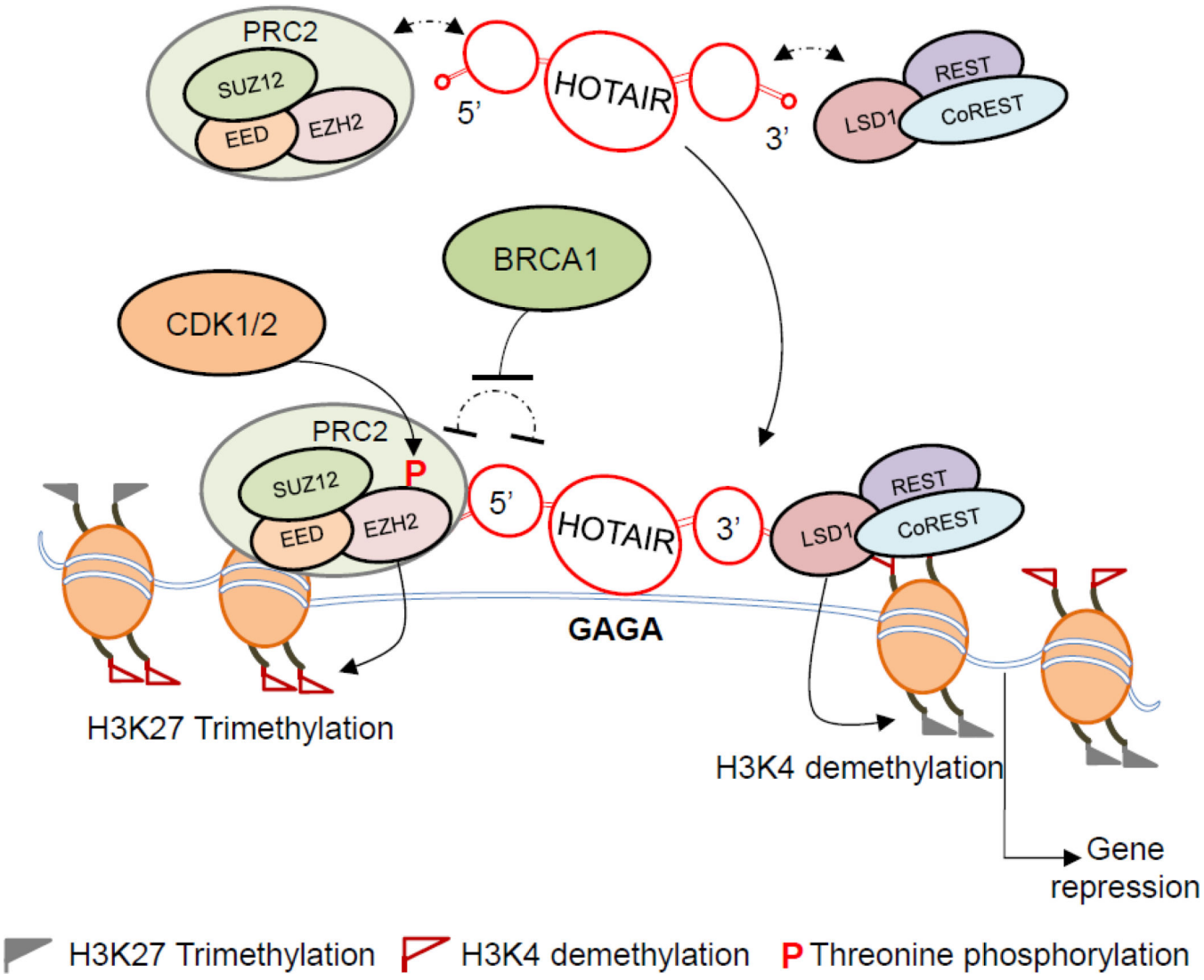

Figure 3.

Mechanism of HOTAIR mediated gene silencing. HOTAIR interacts with PRC2 (that contains EZH2, SUZ12, EEDs and RbAp48) and LSD1 complex (that contains CoREST/REST) via its 5'- and 3'-ends, respectively and recruit them to the target gene promoters/loci. EZH2 introduces H3K27-trimethylation and LSD1 demethylates H3K4 and contribute to gene silencing. BRCA1 and HOTAIR, both interact with PRC2. BRCA1 competes with HOTAIR for binding to PRC2. Thus, expression of BRCA1 inhibits the interaction of HOTAIR with PRC2. CDK1/2 phosphorylates T350 residue (in humans and T345 in mouse) of EZH2 that augments the interaction between EZH2 and HOTAIR.

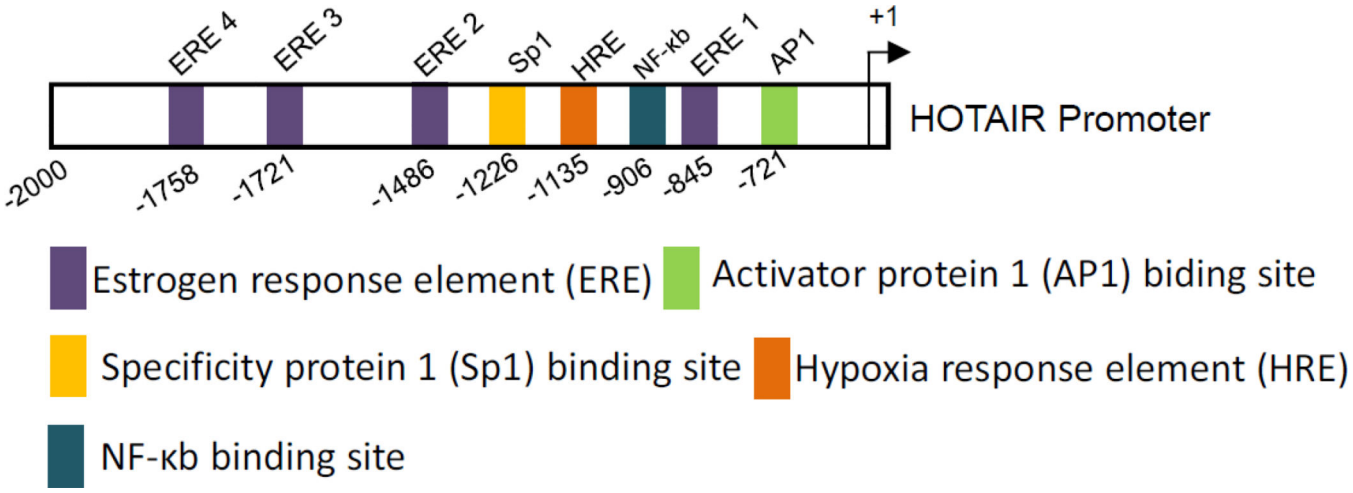

Figure 4.

HOTAIR promoter possesses binding sites for various transcription factors. These include estrogen response elements (ERE), Sp1, AP1, HIF transcription factors, NF-κb and others.

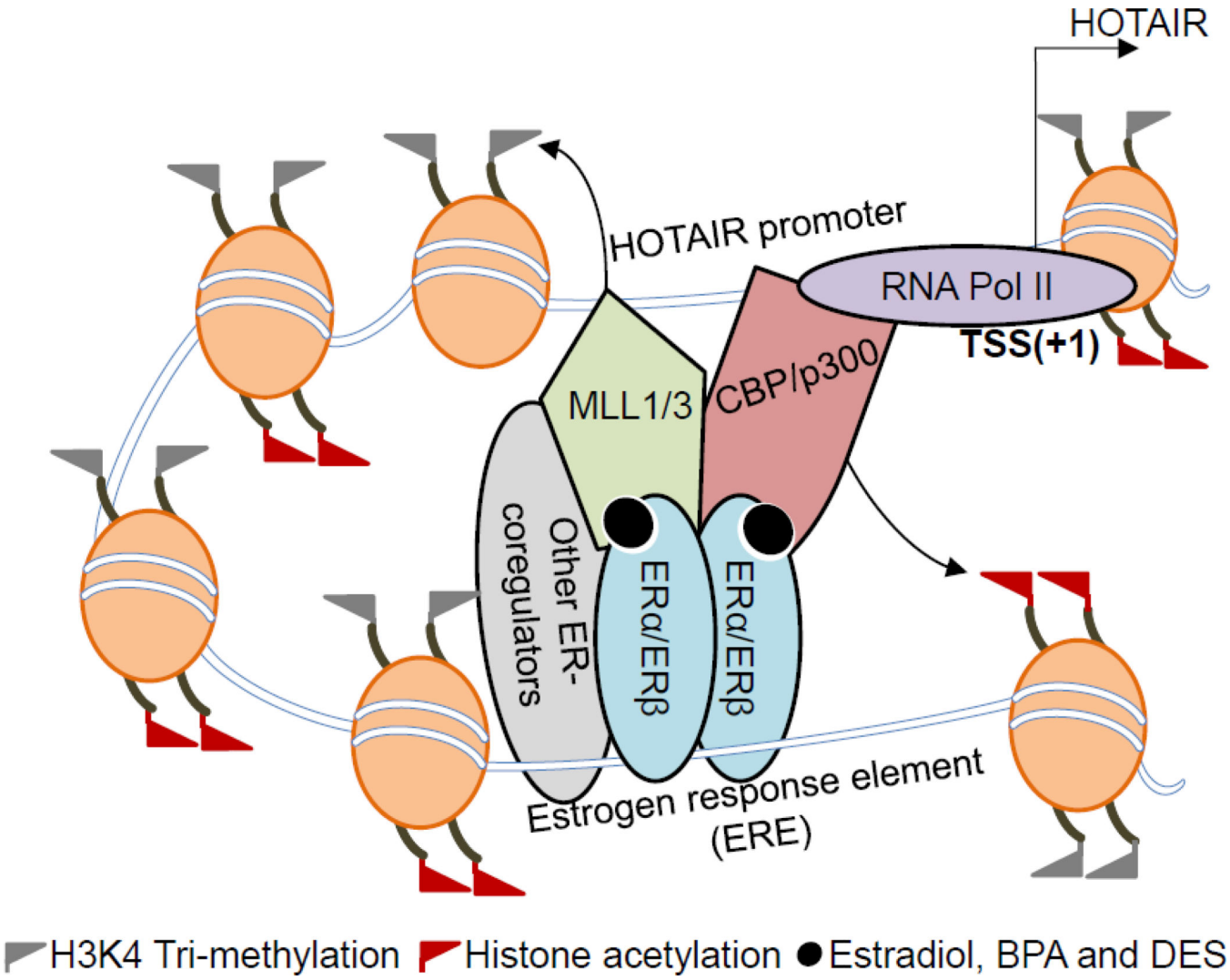

Figure 5.

Model showing the roles of ERs, MLLs and other ER coregulators during by estradiol, BPA and DES mediated transcriptional regulation of HOTAIR. Estradiol and steroidogenic EDCs such as BPA and DES bind to ERs, ERs get dimerized and get activated. Activated ERs bind to the EREs of the HOTAIR promoter. ER coregulators such as MLL-histone methylases (MLL1, MLL3), CBP/p300 (histone acetyl-transferases) and other ER coregulators are also recruited to the HOTAIR promoter. Promoter histones are methylated at H3K4 via MLLs and acetylated via HATs (CBP/p300), allowing chromatin remodeling and access to the RNAP II to the promoter, ultimately resulting in gene activation.

Figure 6.

Regulatory network of HOTAIR. HOTAIR can be induced by estradiol, BPA and DES, Osteopontin, Collagen type-1, NF-κb as well as by other oncoproteins such as c-Myc. On the contrary, miR-141 represses HOTAIR via complementary binding to the HOTAIR and Ago2 mediated degradation of HOTAIR. Epigenetic repression of various genes such as ALPL, BMP2, MMPs, RBM38, p21 etc. by HOTAIR mediated recruitment of PRC2 and LSD1 can regulate various processes such as tumorigenesis, metastasis, Aortic valve calcification drug resistance, cell migration, cell invasion and epithelial to mesenchymal transitions (EMT). HOTAIR also acts a ceRNA and acts as a sink for various miRNAs such as miR-130a, miR-331-3p and miR-124 and modulates various other cellular processes.

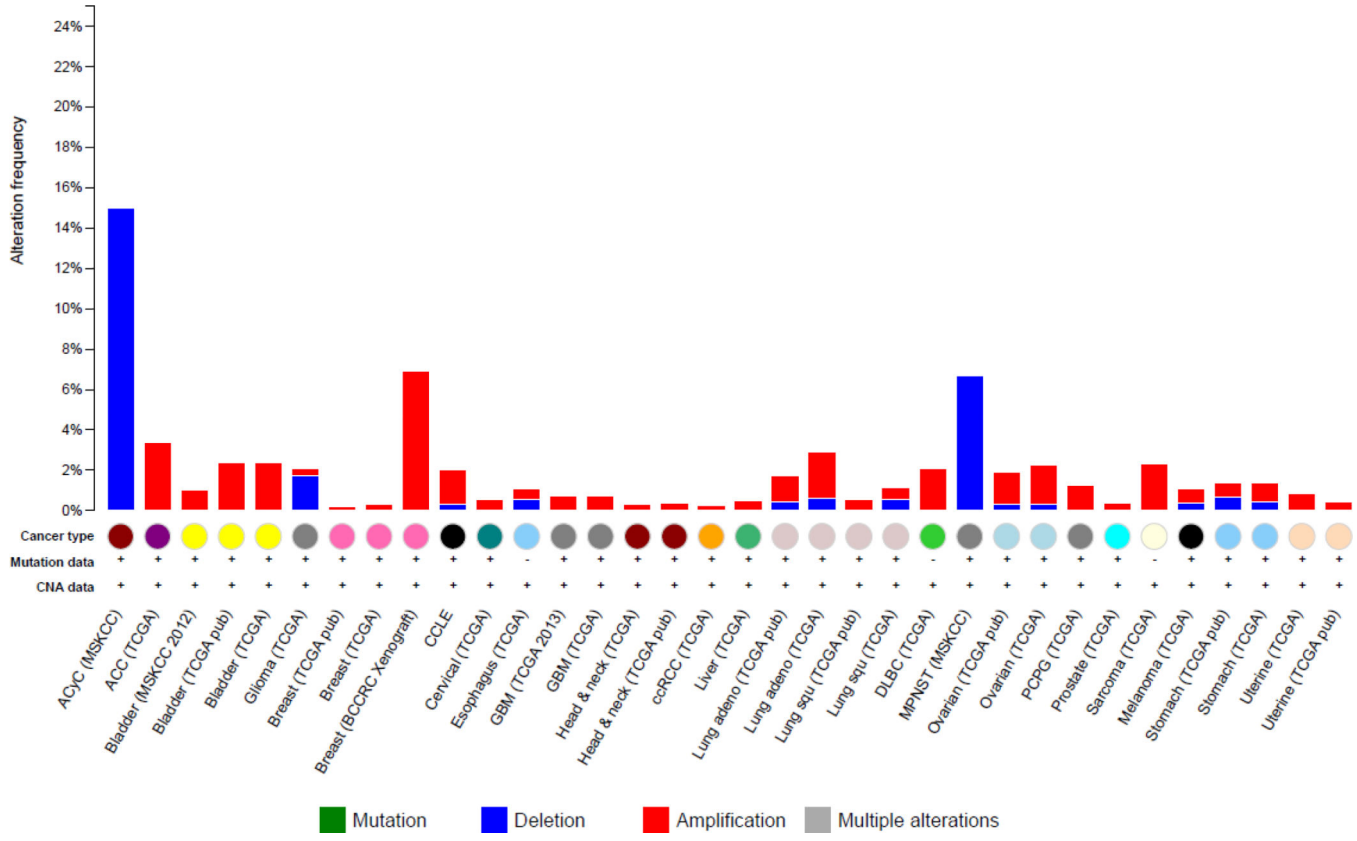

Figure 7.

Cross-cancer summary of copy number alterations and mutations in HOTAIR based on cBioPortal database [150, 151]

Table 1

HOTAIR and its implications in various cancers

Cancer types	**Expression Pattern**	**Type**	**Refs**
Breast cancers	Upregulation	Primary tumors and metastatic tumors	[5, 16, 17, 118, 119]
Hepatocellular carcinoma	Upregulation	Primary tumors and metastatic tumors	[94–97 130]
Colorectal carcinoma	Upregulation	Primary tumors and metastatic tumors	[98, 99, 157]
Gliomas	Upregulation	Primary tumors	[71, 137]
Pancreatic cancers	Upregulation	Primary tumors	[93]
Nonfunctional pituitary adenoma	Upregulation	Primary tumors	[110]
Sarcoma	Upregulation	Primary tumors and metastatic tumors	[102]
Endometrial carcinomas	Upregulation	Primary tumors and metastatic tumors	[117] [68]
Ovarian cancers	Upregulation	Primary tumors	[100, 101]
Esophageal squamous cell carcinoma	Upregulation	Primary tumors	[103–106]
Nasopharyngeal carcinoma	Upregulation	Primary tumors	[107]
Laryngeal squamous cell cancer	Upregulation	Primary tumors	[108]
Non-small cell lung cancer	Upregulation	Primary tumors and metastatic tumors	[131, 133, 135]
Small cell lung cancer	Upregulation	Pure SCLC tumors	[134]
Gastrointestinal stromal cancers	Upregulation	Primary tumors and metastatic tumors	[82]
Gall bladder cancers	Upregulation	Primary tumors	[44]
Prostate cancer	Upregulation	Cell lines	[111]
Melanoma	Upregulation	Primary tumors and metastatic tumors	[114]
Cervical cancer	Upregulation	Primary tumors	[113, 114]
Ta/T1 bladder cancer	Upregulation	Primary tumors	[72]
Gastric cancer	Upregulation	Primary tumors	[45, 115, 116]
Atypical teratoid rhabdoid tumors (ATRTs) such as medulloblastomas, and juvenile pilocytic astrocytomas	Upregulation	Primary tumors	[158]
Ependymomas	Downregulation	Primary tumors	[158]
Urothelial carcinoma	Upregulation	Tumors and cell lines	[92]
Renal carcinoma	Upregulation	carcinoma cells	[20]
Acute myeloid leukemia (AML)	Upregulation	Tumors and cell lines	[51]
Other Diseases	**Expression Pattern**	**Type**	**Refs**
Aortic valve calcification	Downregulation	Bicuspid aortic valve and in human aortic interstitial cells	[152]
Osteoarthritis	Upregulation	Cartilage	[153]
Pre-eclampsia	Upregulation	Placenta and Trophoblast cells	[154]