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Long non-coding RNA and Polycomb: an intricate partnership in cancer biology

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1. ABSTRACT

High-throughput analyses have revealed that the vast majority of the transcriptome does not code for proteins. These non-translated transcripts, when larger than 200 nucleotides, are termed long non-coding RNAs (lncRNAs), and play fundamental roles in diverse cellular processes. LncRNAs are subject to dynamic chemical modification, adding another layer of complexity to our understanding of the potential roles that lncRNAs play in health and disease. Many lncRNAs regulate transcriptional programs by influencing the epigenetic state through direct interactions with chromatin-modifying proteins. Among these proteins, Polycomb repressive complexes 1 and 2 (PRC1 and PRC2) have been shown to be recruited by lncRNAs to silence target genes. Aberrant expression, deficiency or mutation of both lncRNA and Polycomb have been associated with numerous human diseases, including cancer. In this review, we have highlighted recent findings regarding the concerted mechanism of action of Polycomb group proteins (PcG), acting together with some classically defined lncRNAs including *X-inactive specific transcript* (*XIST*), *antisense non-coding RNA in the INK4 locus* (*ANRIL*), *metastasis associated lung adenocarcinoma transcript 1* (*MALAT1*), and *HOX transcript antisense RNA* (*HOTAIR*).

2. LONG NON-CODING RNAs

Transcription is not restricted to protein-coding genes but is pervasive throughout the genome, giving rise to numerous types of non-coding RNA (ncRNA). The first to be identified were infrastructural ncRNAs, such as transfer RNA (tRNA) (1), ribosomal RNA (rRNA) (2), small nuclear RNA (snRNA) (3) and small nucleolar RNA (snoRNA) (4). These ncRNAs play fundamental roles in different cellular processes such as translation (tRNA and rRNA), splicing (snRNA), and rRNA maturation (snoRNA). More recently, regulatory ncRNAs have been discovered as a new class of ncRNA with key roles in cellular homeostasis (5). Among them, an arbitrary size cut-off of 200 nucleotides discriminates two distinct groups: small non-coding RNA (sncRNA) and long non-coding RNA (lncRNA). SncRNAs, such as small interfering RNA (siRNA), microRNA (miRNA) and PIWI-interacting RNA (piRNA), have been thoroughly studied and reviewed (6), and are therefore not the focus of this review. LncRNAs are a large and diverse class of long transcripts (>200 nt) that do not encode proteins. They can be located either intergenically or intragenically, and can be transcribed in both sense and antisense directions. Similarly to messenger RNAs (mRNA), lncRNAs are transcribed by RNA polymerase II and contain an average of 2.29 exons (7), which are typically longer than those found in pre-mRNAs. LncRNAs are 5´capped, undergo splicing and are generally polyadenylated. Despite the similarities shared with mRNAs, lncRNAs are expressed at lower levels and are not evolutionary conserved, which supports the hypothesis that lncRNAs may represent transcriptional noise. However, recent studies have shown that the expression of certain lncRNAs is both dynamic and cell-type-specific during development (8, 9), pointing towards their biological significance. The absence or presence of open reading frames (ORFs) in lncRNAs is an area of debate. Certain studies have found no evidence for protein coding ORFs in lncRNAs (10). However, ribosome profiling studies have provided that lncRNAs may encode short polypeptides (11) that are frequently loaded onto ribosomes (12).

LncRNAs can modulate transcription *in cis*, regulating target genes located at or adjacent to the locus from which they are transcribed. They also function *in trans*, regulating the expression of genes distanced from their host gene on different genomic loci or even in distal chromosomes. The process of being transcribed can be sufficient to enable lncRNAs to regulate the transcription of nearby genes. Indeed, lncRNA expression has been linked to several physiological and pathological pathways, including metabolism, development and cancer, to cite several examples. However, only a few lncRNAs have been well characterized to date, and thus, the mechanism of action of many lncRNAs remains poorly understood.

3. IDENTIFICATION OF lncRNAs-PROTEIN COMPLEXES

LncRNAs can regulate gene expression by interacting with transcription factors and/or chromatin-modifying complexes. Therefore, studying lncRNAs-protein complexes is essential in order to uncover the mechanisms and functions of lncRNAs in biological processes.

As an alternative to classical Ribonucleoprotein Immunoprecipitation (RIP) or RNA pulldown assays, Crosslinking and Immunoprecipitation (CLIP) followed by sequencing (CLIP-seq) have been used to identify new lncRNAs that interact with known RNA-binding proteins (RBPs) (13). CLIP relies on ultraviolet (UV) crosslinking of RNA to RBPs in cells. After partial RNA digestion, ribonucleoprotein complexes are immunoprecipitated and the associated RNAs can be analysed by qPCR or sequencing. Importantly, compared to more recent methods mentioned below, CLIP-seq does not provide full-length sequence information of transcripts, but does enable the mapping of RBP binding sites. CLIP-based methods are limited by: (i) efficiency of UV crosslinking for RNA and protein, (ii) identification of the authentic, crosslinked sequence due to mutations in RNA formed by UV exposure, and, (iii) the requirement for a suitable antibody recognizing a known RBP. Hence, in order

Table 1. Classification of long non-coding RNAs

LncRNA classification	Position to the coding genes	Reference
LincRNAs	Unannotated genomic regions (located between protein coding-genes); *e.g. XIST* or *MALAT1*	(18)
Intronic lncRNAs	Intron of protein-coding genes	(19)
UTR-associated lncRNAs	Sense strand overlapping the 5´UTR or 3´UTR	(21)
Sense lncRNAs	Sense strand exons	(221)
NATs	Antisense strand of protein-coding genes overlapping intronic or exonic regions; *e.g. ANRIL* or *HOTAIR*	(222)
eRNAs	Sense or antisense enhancer sequences	(25)
paRNAs	Sense strand overlapping promoter regions	(223)
PROMPTs	Antisense strand upstream of promoter regions	(31)

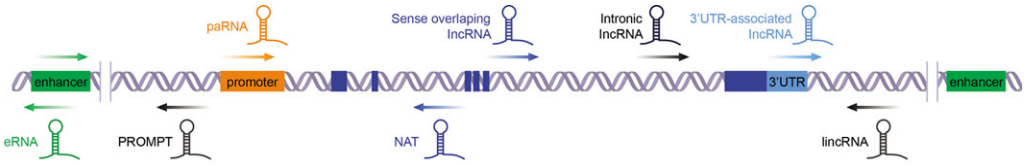

Figure 1. Classification of lncRNA according to the genomic region from which they are transcribed. Enhancer RNAs (eRNAs), promoter upstream transcripts (PROMPTs) and promoter-associated lncRNAs (paRNAs) arise from enhancer regions, upstream regions proximal to promoters and from the promoter associated to protein-coding genes, respectively. Natural antisense transcripts (NATs) originate from the antisense strands while sense overlapping lncRNAs from the sense strands; both can be transcribed from sequences overlapping exons and/or introns. Intronic lncRNAs emerge from introns of protein-coding genes and long intergenic RNAs from regions located between two protein-coding genes. UTRs-associated lncRNAs are transcribed from UTRs of the sense sequence from protein-coding genes. Arrows correspond to the sense or antisense transcription direction

to elucidate new proteins that bind to known lncRNAs, different approaches have been developed.

Capture Hybridization Analysis of RNA Targets (CHART) consists of hybridizing short, affinity-tagged oligonucleotides (biotinylated C-oligos) to target potential genomic binding sites of lncRNAs by hybridization to DNA in crosslinked extracts. By using streptavidin-conjugated beads, both chromatin-associated lncRNAs, as well as the RBPs associated with them, can be identified by either RNase H mapping or mass spectrometry (MS), respectively. The lncRNA *metastasis-associated lung adenocarcinoma transcript 1 (MALAT1)*-associated DNA was identified using the CHART technique (14). Another similar method, Chromatin Isolation by RNA Purification (ChIRP), uses short biotinylated probes (~20 nt) that 'tile' the full-length of lncRNAs to identify their genomic binding sites by sequencing. LncRNA-interacting proteins can also be determined by MS (ChIRP-MS) (15). ChIRP-MS has recently been used to identify 81 proteins that interact with the *X-inactive specific transcript* (*XIST)* lncRNA during embryonic stem cell (ESC) differentiation (16). This MS-based analysis has validated known *XIST* RBPs and identified novel interactors, such as heterogeneous nuclear ribonucleoproteins (HNRNPs) U and K. In comparison to ChIRP, RNA Antisense Purification (RAP) has been developed to retrieve chromatin-lncRNA complexes using longer RNA probes (~120 nt), increasing the specificity of the technique by reducing signal-to-noise ratio (17). Furthermore, lncRNA interacting proteins can be identified by MS (RAP-MS) by using Stable Isotope Labelling by Amino acids in Culture (SILAC), which enables protein quantification. RAP-MS technique has identified 10 proteins that directly interact with *XIST*, including the transcriptional corepressor SMRT/HDAC1 Associated Repressor Protein (17). Thus, the development of these methods that enable both the identification of binding sites of lncRNAs and their associated proteins has immense potential to advance our understanding of gene regulation along with implications in biomedicine.

4. CLASSIFICATION OF LONG NON-CODING RNAs

LncRNAs can be classified according to their relative locations within or adjacent to the protein-coding genes from which they are transcribed (Figure 1 and Table 1): (i) intergenic lncRNAs (lincRNAs), (ii) intronic lncRNAs, (iii) untranslated-region (UTR)-associated lncRNAs, (iv) sense lncRNAs, (v) natural antisense lncRNAs (NATs), (vi) enhancer lncRNAs (eRNAs), (vii) promoter-associated lncRNAs (paRNAs), and (viii) promoter upstream transcripts (PROMPTs).

4.1. LincRNAs

LincRNAs are the class of lncRNAs that have been best studied. They contain the same chromatin signature as actively transcribed genes, namely the K4-K36 domain. This signature consists of a short region with histone H3 lysine 4 trimethylation (H3K4me3) and

a longer region with histone H3 lysine 36 trimethylation (H3K36me3) at the promoter and in the transcribed region, respectively. After excluding K4-K36 domains corresponding to known protein-coding genes, numerous lincRNAs have been identified (18). The expression patterns of certain lincRNAs are correlated to profiles of protein-coding genes, suggesting their conservation throughout evolution. Importantly, these genes are involved in cell-cycle regulation, immune surveillance and pluripotency of ESCs (9), amongst other functions. In addition, it has been reported that lincRNAs can interact with chromatin remodelling proteins, *e.g.* Polycomb group of proteins (PcG), in order to regulate gene expression.

4.2. Intronic lncRNAs

Intronic lncRNAs are the most abundant lncRNAs. It has been demonstrated that the expression profile of intronic lncRNAs and their cognate protein-coding gene correlate and possess tissue-specific patterns (20). Newly identified circular intronic lncRNAs (ciRNA) are derived from spliced introns that do not undergo debranching. CiRNA are abundant in the nuclei and have been suggested to sequester RBPs to regulate gene expression (20).

4.3. UTR-associated lncRNAs

UTR-associated lncRNAs can be transcribed independently of the transcription of the parental gene. However, since they overlap with each other, it has been challenging to study the function of UTR-associated lncRNAs through knockdown or knockout strategies (21). The expression of UTR-associated lncRNAs has been shown to be cell-type specific and they can act as decoys to regulate the binding of transcription factors.

4.4. Sense lncRNAs

Sense lncRNAs may contain a small ORF (sORF) overlapping with the same start codon as their host gene, thus encoding a small polypeptide. Sense lncRNAs can overlap with protein-coding genes or with entire introns. Numerous sense lncRNAs have been shown to function both as ncRNAs and protein-coding genes, for example *SRA*, *p53* and *ENOD40* (22).

4.5. NATs

NATs overlap with protein-coding genes and are transcribed from the opposite strand at the same genomic locus. They function either in *cis* to regulate the transcription of the sense protein-coding gene, or in *trans* to regulate the transcription of genes located at different genomic loci. NATs undergo fewer splicing events and are less abundant than their corresponding sense transcripts. Even though NATs are found in animals, plants, yeast, and prokaryotes (23), they are not evolutionary conserved.

4.6. eRNAs

eRNAs are known to be involved in chromatin looping to stabilize the interaction between enhancer and promoter of a nearby target gene, which promotes transcriptional initiation. They play important roles in genome integrity, cellular homeostasis and metabolic responses (24, 25). eRNAs originate from enhancer regions which are particularly enriched with the histone H3 lysine 27 acetylation (H3K27ac) mark (26, 27).

4.7. paRNAs

paRNAs are transcribed from the sense or antisense direction in correlation of the promoter of a protein-coding gene. paRNA lncRNAs can directly inhibit the transcription of a nearby protein-coding gene through direct alteration of the chromatin state (28), or by the recruitment of other proteins, such as transcription factors (29). For instance, a *cyclin D1* (*CCND1*) paRNA has been found to recruit an RBP translocated in liposarcoma (TLS) to the promoter of *CCND1* to exert transcriptional repression through histone acetyltransferase inhibitory activity (30).

4.8. PROMPTs

PROMPTs arise from the antisense strand in nucleosome-depleted regions. Previous studies have shown that PROMPTs are 5' capped and 3' polyadenylated (31), however rapidly degraded by the nuclear exosome. Therefore, it remains unclear whether they have a cellular function, particularly because they are located in nucleosome-depleted regions that expose DNA to binding factors, which could randomly initiate transcription.

This wide range of lncRNA that have been defined thus far appear to function in intricate regulatory networks that usually involves epigenetic machineries. Their dysregulation is a hallmark of many diseases, including cancer.

5. FUNCTIONS OF lncRNAs

Most lncRNAs that have been characterized play important roles in regulating gene expression by interacting with DNA, RNA and protein complexes, and can function in both the nucleus and cytoplasm. Here, we briefly describe some possible mechanisms by which lncRNAs act at multiple levels to regulate gene expression (Figure 2)

5.1. Signal

The expression of a lncRNA is cell type-specific and varies in response to specific stimuli,

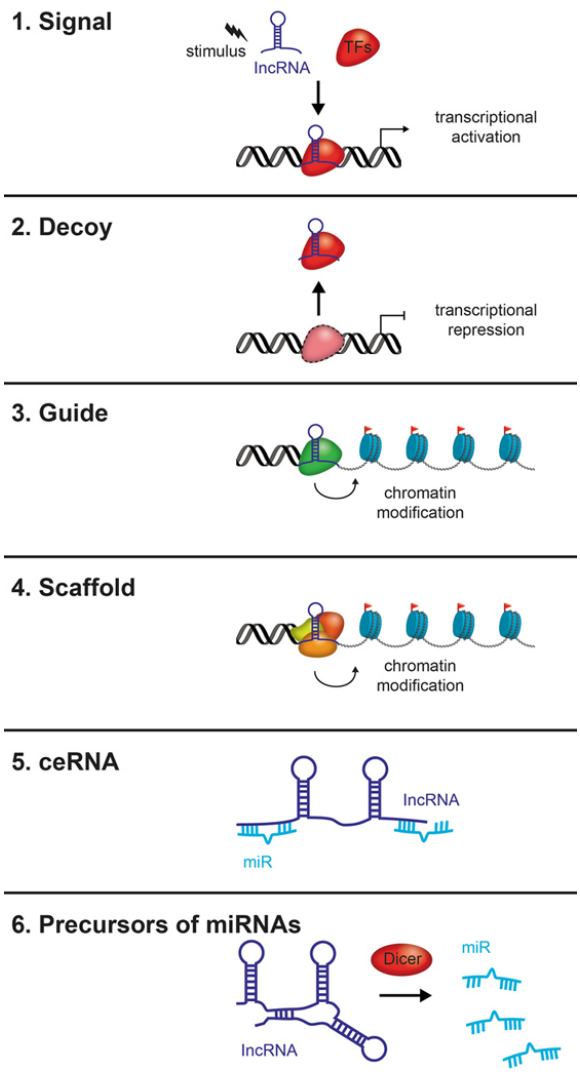

Figure 2. Functions of lncRNAs in regulating gene expression. (1) LncRNAs can act as a molecular signal and activate the transcription of protein-coding genes in a stimulus-dependent manner. (2) LncRNAs can repress the binding of transcription factors (TFs) by binding to their DNA-binding domain. (3) They can also serve as guides for chromatin- remodelling proteins by recruiting these proteins to specific loci; (4) or as scaffold to assemble chromatin-remodelling complexes. (5) In the competitive endogenous RNA (ceRNA) hypothesis, miRNAs can bind to lncRNAs (miRNA sponging) instead of their target genes which in turn activate the expression of the target genes. (6) LncRNAs can also serve as precursors of miRNAs, which involves the cleavage of lncRNAs by the endonuclease Dicer.

indicating that it is under transcriptional control. Because some lncRNAs possess a regulatory function, they can serve as a molecular signal, *e.g.* to integrate developmental cues (32).

5.2. Decoy

A lncRNA can inhibit the function of a protein, such as a transcription factor or a chromatin modifier, by acting as a binding site and thereby titrating away a protein target (33, 34).

5.3. Guide

A lncRNA can recruit chromatin-remodelling proteins to specific loci to activate or inactivate transcription (35).

5.4. Scaffold

Because of their secondary structure, lncRNAs can bind more than two protein partners, where the lncRNA serve as adaptors to assemble regulatory protein complexes onto chromatin (36).

5.5. miRNA sponge

Recent studies have proposed the competitive endogenous RNA (ceRNA) hypothesis, in which transcripts with common miRNA binding sites can inhibit miRNAs competitively, by acting as a molecular 'sponge', leading to the de-repression of miRNA targets (37, 38). Nevertheless, the prevalence, functional significance and targets of lncRNAs as miRNA sponges in cancer development are mostly unknown.

5.6. miRNA precursor

LncRNAs can be processed to generate miRNAs, pointing towards an interplay in gene expression regulation between the lncRNA and miRNA pathways (39, 40).

6. POST-TRANSCRIPTIONAL MODIFICATIONS OF lncRNAs

Recent methylated RNA sequencing studies have shown that RNA modifications are present in lncRNAs, and such modifications could affect processing, stability, intracellular localization, as well as their interactions with proteins or RNA targets (for review see 41). N^6-methyladenosine (m^6A) is the most abundant internal modification in mRNAs and lncRNAs. Previous work has identified the presence of two m^6A residues in the *MALAT1* lncRNA that are located in two hairpin-stem structures (42). One of these modified nucleotides (A2577) reduces the stability of the hairpin stem, influencing the accessibility of a protein binding site by a mechanism termed the "m^6A-switch" (43). Specifically, m^6A modification disrupts base-pairing and exposes the opposing U-rich site for the binding of heterogeneous nuclear ribonucleoprotein C (HNRNPC). Similarly, the binding of heterogeneous nuclear ribonucleoprotein G (HNRNPG) is enriched when *MALAT1* is m^6A-modified (44). m^6A modification is also involved in X chromosome inactivation. *XIST* lncRNA is highly decorated with m^6A, which acts as a scaffold for the assembling of silencing complexes, such as YTH domain containing 1 (YTHDC1), for *XIST*-mediated gene repression (45). LncRNAs can also bear 5-methylcytosine (m^5C) modifications

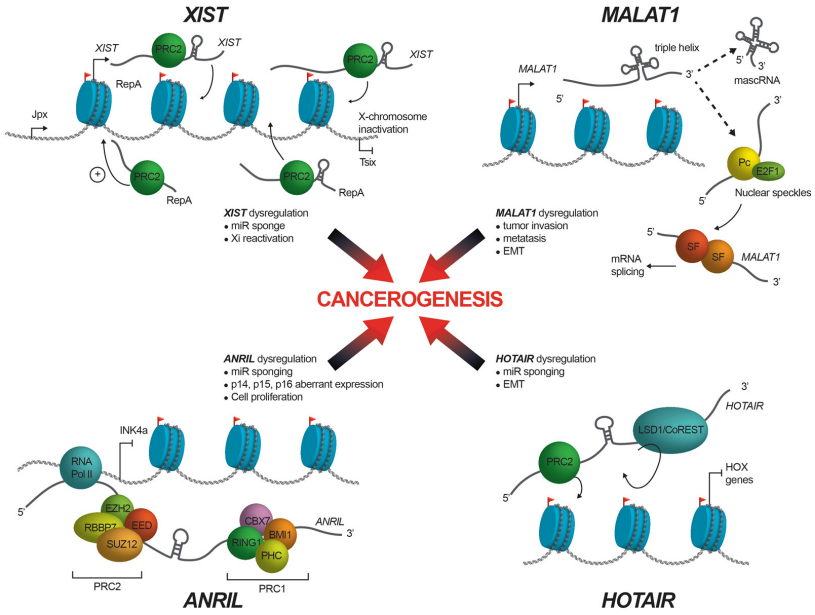

Figure 3. LncRNAs are associated to Polycomb. (Upper left) During the X chromosome inactivation (XCI), *X-inactive specific transcript* (*XIST*) recruits Polycomb Repressive Complex 2 (PRC2) to the X inactivation center (XIC) to silence genes expression, which is propagated along the inactive X chromosome (Xi). In human cancer, *XIST* overexpression can be associated with miRNA (or miR) sponging while *XIST* loss can be associated with Xi reactivation. (Upper right) *Metastasis associated lung adenocarcinoma transcript 1* (*MALAT1*) is processed to generate *MALAT1-associated small cytoplasmic RNA* (mascRNA) and a longer transcript. The longer transcript is retained in nuclear speckles and interacts with splicing factors (SF) to regulate mRNA splicing. *MALAT1* overexpression is associated with epithelial-to-mesenchymal transition (EMT) and can be involved in miR sponging in certain types of cancer. Loss of *MALAT1* is associated with apoptosis and reverses the tumorigenic phenotype. (Lower left) *Antisense non-coding RNA in the INK4 locus* (*ANRIL*) recruits PRC1 and PRC2 *in cis* to repress the transcription of *INK4b* (p15) and *INK4a* (p16). Loss of *ANRIL* increases p15 and p16, which inhibits cell proliferation. In cancerous cells, *ANRIL* can also act by miR sponging to activate *E2F1*. (Lower right) *HOX transcript antisense RNA* (*HOTAIR*) recruits PRC2 at its 5' end and LSD1 at its 3' end to repress *HOXD* genes. Overexpression of *HOTAIR* in cancer triggers the interaction of SUZ12/PRC2 and LSD1 to target tumour suppressor genes, whereas depletion of *HOTAIR* releases SUZ12/PRC2 and LSD1 from their target genes.

(46). Loss of m^5C within eRNAs diminishes their stability, affecting the transcription of associated mRNAs (24). Another study has demonstrated the presence of m^5C in *HOTAIR* and *XIST* lncRNAs within functionally important regions that are known to mediate interaction with chromatin remodelling complexes (46). For instance, bisulfite sequencing of RNA has identified the m^5C mark within the repeat-A region of *XIST,* which affects Polycomb repressive complex-2 (PRC2) binding (46). Furthermore, N^1-methyladenosine (m^1A) and pseudouridine (Ψ) sequencing approaches have also identified these modifications on lncRNAs such as *MALAT1* and *XIST* although their function remains unclear (47). Hence, further studies are needed in order to understand the functional relevance of lncRNA modification during carcinogenesis.

7. POLYCOMB GROUP OF PROTEINS

PcG were originally characterized in *Drosophila melanogaster* as transcriptional repressors of homeotic genes (Hox), which are required for the correct spatiotemporal expression of developmental regulators along the body axis (48). In most metazoan species, PcG complexes include PRC1, PRC2 (49), and the more recently identified Pho-Repressive Complex (Pho-RC) and Polycomb Repressive De-Ubiquitinase (PR-DUB) (50). In *D. melanogaster*, PcG are recruited to specific target sites by Polycomb response elements (PREs), 1 kb DNA elements containing recognition sequences for DNA binding proteins. So far, PREs have not been elucidated in mammalian, remaining unclear how PRC1 and PRC2 are targeted onto chromatin to identify their target genes.

The minimal PRC2 core complex consists of three subunits: Enhancer of Zeste 1/2 (EZH2/1), Embryonic Ectoderm Development (EED), and Suppressor of Zeste 12 (SUZ12). EZH2/1 contain a putative RNA-binding domain and a conserved SET domain that catalyses the mono-, di-, and trimethylation of lysine 27 (K27) of histone H3 (H3K27me1, H3K27me2, and H3K27me3), contributing to the repressive activity of PRC2 (51-54). SUZ12 is a regulatory subunit with a putative RNA-binding domain whereas EED binds H3K27me3 to increase the affinity of PRC2 with nucleosomes. Both SUZ12 and EED are required for EZH2 enzymatic activity.

After imposition of H3K27me3 by PRC2, PRC1 is recruited and catalyses the ubiquitylation of histone H2A on K119 (H2AK119Ub), maintaining transcriptional repression (55). The core complex of

Table 2. Association of *XIST*, *ANRIL*, *MALAT1*, and *HOTAIR* lncRNAs with Polycomb

LncRNA	Polycomb	Subunits	Reference
XIST	PRC2	EZH2 SUZ12 RBP4 + RBP7	(71, 106) (107) (224)
	PRC1	RYBP PCGF3/5	(16, 115, 224) (16, 113)
ANRIL	PRC2	SUZ12 EED RBAP46	(144, 138) (144) (144)
	PRC1	CBX7 RING1B	(73) (144)
MALAT1	PRC2	EZH2 SUZ12	(173, 173) (174)
	PRC1	CBX4	(82)
HOTAIR	PRC2	EZH2	(76, 106, 211)
	PRC1	SUZ12	(76)

PRC1 presents four subunits that are heterogeneous depending on the cellular context. In mammals, these subunits are homologous to those found in *D. melanogaster* (56) and consist of; (i) a subunit that belongs to the chromobox family (CBX2, CBX4, CBX6, CBX7, or CBX8) containing a chromodomain which binds to H3K27me3; (ii) a Polyhomeotic-like protein (PHC1, -2 or -3); (iii) the E3 ubiquitin-protein ligase RING1A/B; and (iv) a member of the PcG RING finger (PCGF) family including BMI1 (also known as PCGF4) or MEL18 (also known as PCGF2). PCGF proteins catalyse the formation of H2AK119Ub, which not only modulate transcriptional elongation by Pol II but also the formation of preinitiation complexes (57, 58). How PRC1 functions to repress transcription is not well understood as numerous PRC1 components have been described, increasing the complexity of study. Recently, non-canonical forms of PRC1 have been characterized. All PCGF proteins can interact with RYBP/YAF2 in competition with CBX proteins, forming a variant of PRC1 devoid of CBX proteins. Studies have shown that a variant of PRC1 (containing PCGF1, 3, 5) is recruited to chromatin first and is responsible for H2A ubiquitylation, independently of PRC2 recruitment and H3K27me3 deposition (59). Notably, the variant PCGF1/PRC1 complex contains an additional factor, KDM2B, which is a H3K4 and H3K36 lysine demethylase. KDM2B is recruited to non-methylated CpG islands via its DNA-binding domain, and ultimately facilitates the recruitment of PRC2, required for the deposition of H3K27me3.

Importantly, PcG complexes modulate gene expression during differentiation, cell lineage specification and morphogenesis (60, 61). Loss of PRC1/2 subunits have been shown to lead to embryonic lethality (62, 63), and mutations in PcG proteins can lead to altered activity, which might promote aberrant expression of oncogenes (64, 65). For instance, the overexpression of PRC1 subunit BMI1 is associated with a repression of the tumour suppressor locus *INK4b-ARF-INK4a*, which in turn induces cell proliferation (66). In addition, previous studies have shown that the overexpression of EZH2 is involved in several cancers, such as melanoma, breast and prostate cancer (67-69). Therefore, it is important to gain a deeper understanding of the interplay between lncRNA and PcG in order to elucidate their combined role in cancer initiation and progression.

8. POLYCOMB RECRUITMENT BY lncRNAs

Several lncRNAs have been shown to recruit Polycomb proteins to specific loci in order to modify epigenetic chromatin states, and thereby to repress gene expression. Some well-documented examples include *XIST* (70, 71), *antisense non-coding RNA in INK4 locus* (*ANRIL)* (72, 73)*, MALAT1* (74, 75), and *HOX transcript antisense intergenic RNA* (*HOTAIR)* (76) (Table 2), which will be extensively described below.

Other examples of lncRNAs that recruit PcG proteins to exert their biological function include *H19*, *KCnqt1ot1* and *Air* (to imprinted genes), *Braveheart* (during cardiomyocyte differentiation), *Meg3* (in pluripotent stem cells) (77), and *PINT* (which is regulated by p53), among others. Indeed, a significant proportion of all lncRNAs (~20%) is found in association with PRC2 in human cell lines (18). Depletion of PRC2-associated lncRNAs, such as *HOTAIR* and *TUG1*, resulted in the activation of PRC2 target genes. Following this discovery, RIP-seq analysis identified ~10,000 lncRNAs associated with PRC2 in ESCs, belonging to antisense, intergenic and promoter-associated categories along with unannotated lncRNAs (78). These transcripts emerged from imprinted regions, containing oncogenes or tumour suppressor genes, suggesting that lncRNA-mediated recruitment of PRC2

influences carcinogenesis. *In vivo*, the interaction between lncRNAs and PRC2 could occur through multiples mechanisms, including bridging of regulatory proteins, and can be influenced by post-translational modification of chromatin remodelling complexes, or lncRNAs. It has been shown *in vitro* that the interaction between lncRNAs and PRC2 occurs through the EZH2 subunit. However, high affinity PRC2 binding does not rely on a specific RNA sequence but rather appears to be promiscuous. Indeed, recent studies have shown that PRC2 does not necessarily discriminate between RNA species. For example, PRC2 can bind to both *in vitro* transcribed RNA from the 5' end of *HOTAIR* and to maltose-binding protein (MDB) mRNA from *Escherichia coli*, which lack Polycomb proteins. This supports the concept that PRC2 binding to lncRNA is unspecific (79). On the other hand, a recent study has shown that RNA and chromatin interact competitively with PRC2 (80). As such, degradation of RNAs increased the recruitment of PRC2 to chromatin, whereas the release of PRC2 from chromatin increased RNA binding to PRC2. Although there has been considerable focus on PRC2, several studies have shown that lncRNAs can also bind to PRC1, in particular to CBX proteins. CBX7 directly interacts with ANRIL, resulting in repression of the *INK4b/ARF/INK4a* tumour suppressor locus (81). Furthermore, CBX4 binds to TUG1 and MALAT1/NEAT2, stimulating E2F1 SUMOylation *(82),* which results in increased cellular proliferation. Nevertheless, the nature of interactions between lncRNAs and PcGs *in vivo* remain unclear. Hence, a major unanswered question is how lncRNAs recruit PcG to specific targets.

9. *XIST*

XIST is a lncRNA that plays a central role in the initiation of X chromosome inactivation (XCI) in early embryogenesis (83-85). This process leads to the highly regulated transcriptional silencing of one X chromosome in female mammals (designated as inactive X-chromosome (Xi) or Barr body (86)) to ensure dosage compensation between the sexes (87, 88). In mice, XCI occurs in two lineage-specific forms. At the 2-4-cell stage, imprinted XCI leads to parental X-chromosome silencing, and the paternally imprinted Xi is sustained in the cells of the trophectoderm and primitive endoderm, which give rise to extra-embryonic tissues (89, 90). In contrast, Xi is re-activated in the epiblast of the inner cell mass, and then random XCI is induced during the peri-implantation stage. Thus, both the paternal and maternal X chromosomes have an equal chance of being inactivated.

The *XIST* gene is located in the X inactivation centre (XIC) (91, 92), and its expression marks the future Xi. Two RNA-based switches positively and negatively regulate *Xist*: *Jpx* for Xi, and *Tsix* for active X-chromosome (Xa). *Jpx* RNA may bind CTCF and titrate out its repressive effect on the *Xist* promoter (32). Conversely, *Tsix* represses *Xist* induction by several means, including altering the chromatin state of *Xist* (93-95), recruiting the RNAi machinery (96), and facilitating PRDM14 binding to *Xist* intron 1 to suppress its expression (97). In turn, *Tsix* is regulated by *Xite*, a proximal ncRNA that sustains *Tsix* expression on the future Xa (98, 99).

9.1. *XIST* and Polycomb

Chromosome coating by *Xist* recruits chromatin modification complexes that initiate gene silencing on the Xi, which will be propagated as Xi in all subsequent cell divisions throughout the life of the female individual (88). A key silencing factor recruited by *Xist* is Polycomb (100). Both PRC2 and PRC1 complexes are enriched on the Xi early on during differentiation, but become depleted at later stages, suggesting that their association with the Xi is linked to early maintenance (101, 102). Moreover, PRC2/H3K27me3 have been shown to co-localize with *XIST* lncRNA, both at metaphase and interphase (70, 103, 104). During XCI, PRC2 is first targeted to the *Xic* by *RepA*; a lncRNA located within the 5' end of *Xist* that encompasses the conserved A-repeat domain of *Xist* (71, 105). *Xist* then recruits PRC2 (101, 103, 104) through direct interaction between the A-repeat domain and EZH2 and/or SUZ12 (71, 106, 107) or indirectly through JARID2 (108). From *Xic*, PRC2 spreads along the future Xi, concentrating predominantly within bivalent domains coinciding with CpG islands (109). As XCI proceeds, the coating of the future Xi by *Xist* lncRNA correlates with recruitment of 3,000-4,000 moderate Polycomb sites, facilitating the spreading of H3K27me3. Nevertheless, the aforementioned model has been challenged by non-confirmatory results. For example, *Xist* expression in early mouse embryos precedes PRC2 recruitment to Xi (90, 110), and *Xist* lacking the A-repeat domain is able to recruit PRC2, albeit less efficiently (101). Moreover, super-resolution FISH/immunofluorescence studies have shown that PRC2 and *XIST* lncRNA are spatially segregated (111). A further consideration is that comprehensive identification of RBPs by MS has not identified PRC2 as an interactor with *Xist*, although PRC1 proteins were detected (16, 17).

It has been shown that PRC1 also participates in XCI (112). Although it was originally thought that PRC1 functioned through the classical hierarchical model described above, a novel mechanism referred as reverse hierarchical recruitment has been proposed whereby PRC1 recruitment in Xi precedes that of PRC2 (113). Indeed, H2AK119Ub imparted by PRC1 was present on Xi in the absence of PRC2/H3K27me3 (114). Furthermore, non-canonical PRC1 complexes (containing the RYBP cofactor) are also recruited to Xi, *via* an *XIST* lncRNA dependent but H3K27me3 independent mechanism (59, 115).

9.2. Role of XIST in tumorigenesis

Although XCI occurs during early development, *XIST* is also expressed in adult females. Several studies have shown alterations of XCI in human cancer. In aggressive breast and ovarian tumours, the Barr body is frequently undetectable (116-119). More recently, cytogenetic studies of breast carcinoma provided evidence that the loss of the Barr body can be accompanied by the gain of an additional Xa, which may be due to X-linked gene reactivation (120-122). The role of X chromosome dosage in breast tumour development is further strengthened by evidence from male breast tumours, where lack of the Y and a duplication of the X chromosome are often observed (123, 124). In addition, males with supernumerary Xs have an increased risk of developing breast cancer (125).

Several studies suggest that loss of *XIST* lncRNA could drive disease progression in cancer through reactivation of Xi. Indeed, *XIST* lncRNA has been shown to act as a tumour suppressor by reducing activation of the AKT pathway in breast cancer, resulting in limited cell viability. Knockdown of either *XIST* or *SPEN*, encoding an important interactor for XCI establishment (16, 17), suppressed the expression of phosphatase PHLPP1 in AKT dephosphorylation (126). In addition, RNA expression profiling of sporadic basal-like cancers, which have lost Xi and *XIST* lncRNA, also displayed overexpression of some X-linked genes (127). Loss of *BRCA1*, a tumour suppressor protein that is frequently mutated in familial cases of breast cancer (128), was initially found to lead to Xi perturbation and dysregulation of *XIST* lncRNA (129). Subsequently, *BRCA1* was shown to maintain proper Xi heterochromatin (130). Furthermore, loss of *XIST* lncRNA was associated with a *BRCA1* deficiency in sporadic basal-like cancers (127). However, subsequent studies revealed that *XIST* lncRNA functioned independently of *BRCA1* in XCI (131-133). Another chromatin regulator frequently overexpressed in cancer, Aurora B Kinase (AURKB), has also been proposed to regulate the association of *XIST* to the Xi (134). However, the precise consequence of *XIST* on the status of Xi is unclear. Direct causality has now emerged from a recent study showing that loss of *XIST* in the hematopoietic lineage led to the development of female-specific leukemia in mice (135). Gene expression profiling over the course of disease progression revealed significant upregulation of X-linked genes, suggesting the possibility of Xi reactivation following *XIST* loss. This sensitivity of hematopoietic cells to *Xist* dysregulation support previous work in which overexpression of *Xist* in mice resulted in lethal anemia due to defective haematopoiesis (136), and overexpression in a lymphoma cell model suppressed tumorigenicity (137).

10. *ANRIL*

ANRIL (also known as *CDKN2B antisense RNA 1* (*CDKN2B-AS1*)) is transcribed in the opposite direction from the *INK4b-ARF-INK4a* tumour suppressor locus and the *methylthioadenosine phosphorylase* (*MTAP*) gene (138, 139). The *INK4b-ARF-INK4a* locus encodes three critical tumour suppressor genes, *p14ARF* (*p19ARF* in mice), *p15INK4b*, and *p16INK4a*, all of which play a central role in cell cycle arrest, affecting key cellular processes such as senescence, apoptosis, and ESC self-renewal by triggering both retinoblastoma (Rb) and p53 pathways (140, 141). The locus also contains a fourth gene, *MTAP*, which has been associated with carcinogenesis (142, 143). *ANRIL* contains 19 exons, many of them consisting of LINE, SINE, and Alu repetitive elements (144,145). The first exon of *ANRIL* is located 300 nt upstream of the transcription start site of *ARF*. Hence, these two genes share a bidirectional promoter (146, 147). Moreover, *ANRIL* can exist in both linear and circular (circANRIL) forms, which have been reported to be cell- or tissue-specific (144, 148-151), yet their functional relevance is still unknown.

10.1. *ANRIL* and Polycomb

ANRIL functions as a cis-regulator of the *INK4a-ARF-INK4b* locus by recruiting PRC1 and PRC2 complexes (81, 138). *ANRIL* has been shown to interact with the PRC2 component SUZ12 to repress the expression of *p15INK4b*. Thus, depletion of *ANRIL* increased the expression of *p15INK4b* and inhibited cellular proliferation (138). Moreover, it has been shown that *ANRIL* is stably associated with PRC1 *via* CBX7 as a nascent transcript generated by the Pol II. This allowed CBX7 recognition of H3K27me3, leading to *p16INK4a* silencing (81). In line with these findings, RIP-seq experiments in which two specific exon-combinations of *ANRIL* were overexpressed, showed a binding of *ANRIL* with CBX7 and RING1B of PRC1, binding with the PRC2 subunits EED, RBAP46 (also known as RBBP7), and SUZ12, and PRC-associated proteins JARID2, RYBP and YY1 (144).

10.2. Role of *ANRIL* in tumorigenesis

ANRIL was originally identified in familial melanoma patients with neural system tumours and a germ-line deletion of the entire *INK4b-ARF-INK4a* gene cluster, suggesting that *ANRIL* might play a role in oncogenesis (152). The gene cluster has been shown to be deleted or silenced in both alleles in ~40% of human cancers (153). On the other hand, genome-wide association studies have identified *ANRIL* as a risk factor in several types of cancer, including gastric cancer, esophageal squamous cell carcinoma, breast and bladder cancers etc. (154-157). Moreover,

the *ANRIL* locus has been identified as a hotspot for disease-associated polymorphisms showing a significant correlation with tumour development, cardiovascular disease, and other conditions (158). These polymorphisms alter the expression pattern of *ANRIL* splice variants, and in consequence dysregulate the *INK4b-ARF-INK4a* locus expression.

In normal cells, induction of *ANRIL* transcript levels by E2F1 is required for the suppression of *p14ARF*, *p15INK4b*, and *p16INK4a* expression at the late stage of the DNA damage response, in order to return to baseline levels after the completion of the DNA repair process. However, in cancerous cells, aberrant expression of *ANRIL* would cause a blockage DNA damage response control, leading to genomic instability and tumour progression (159). It has been shown that *ANRIL* also influences cell proliferation by regulating target genes *in trans*. Hence, in gastric cancer tissues, *ANRIL* cooperated with miRNAs in the epigenetic level by binding to EZH2. Specifically, *ANRIL* silenced miR-99a/miR-449a, which triggers the activation of miR-99a/miR-449a target genes *mTOR* and *CDK6*, and as a consequence upregulated the *CDK6* target gene *E2F1* (156). Similarly, in hepatocellular carcinoma *ANRIL* abolished miR-122-5p expression, enhancing colony formation ability, metastasis and invasion (160). Moreover, in esophageal squamous and thyroid cancer cells, *ANRIL* has been shown to influence cell growth by repression of the TGFβ/Smad signalling pathway (154, 161), although the nature of the interaction between *ANRIL* and TGFβ1 is unclear.

11. MALAT1

MALAT1 (also known as *nuclear-enriched transcript 2* (*NEAT2*)) was originally found to be associated with lung cancer (162). Although *MALAT1* is a highly abundant lncRNA conserved across many mammalian species, it is not essential for mouse development (163). *Malat1* knockout mice do not show any abnormality during embryonic or postnatal development, suggesting that *MALAT1* is dispensable or might be important only under certain pathological conditions (163-165).

MALAT1 can be found as a long nuclear transcript or as a small cytoplasmic RNA. Thus, the primary *MALAT1* transcript contains a tRNA-like structure at its 3´ end, which is cleaved by enzymes involved in tRNA biogenesis. It is processed to form the *MALAT1-associated small cytoplasmic RNA* (mascRNA), which might fulfil additional, but so far unknown, functions (166). The 3' end of *MALAT1* contains a conserved triple-helix structure termed expression and nuclear retention element (ENE), which prevents 3' end nuclease cleavage and enhances translation when placed downstream of an ORF (167, 168).

At the molecular level, multiple functions have been proposed for *MALAT1 (139)*. The long *MALAT1* transcript is retained in the nucleus and specifically localizes to nuclear speckles where it interacts with several pre-mRNA splicing factors to regulate alternative mRNA splicing (169, 170). *MALAT1* is not essential for the integrity of nuclear speckles but for the modulation of the association of active pre-mRNA splicing factors with speckles (170). It has also been linked to the modulation of the epigenetic machinery (171), and is known to be associated with active genes where it would serve as a scaffold that binds proteins to activate transcription at specific loci (171).

11.1. MALAT1 and Polycomb

MALAT1 has been shown to promote tumour cell proliferation, invasion and metastasis through Polycomb. In T and NK cell lymphoma, *MALAT1* lncRNA is highly expressed and this has been correlated with the PRC1 component BMI1, and related to poor prognosis in patients with mature T cell lymphoma. In addition, direct binding of *MALAT1* to the PRC2 components EZH2 and SUZ12 was shown in a T cell lymphoma cell line (172). In renal carcinoma, *MALAT1* interacted with EZH2 to promote Wnt/β-catenin pathway, thus promoting tumour invasion and metastasis (173). In addition, *MALAT1* has been shown to facilitate epithelial-to-mesenchymal transition (EMT) through interaction with SUZ12, which resulted in downregulation of E-cadherin and upregulation of N-cadherin and fibronectin (174). In association with the PRC1 subunit CBX4 (E3 SUMO-protein ligase) and other protein complexes, *MALAT1* controls sub-nuclear architecture, acting as a sensor for the activation of specific transcriptional programs. When methylated CBX4 is bound to the *TUG1* lncRNA, it can be recruited to growth control genes within repressive Polycomb bodies. In response to growth signals, the demethylated form of CBX4 interacts with *MALAT1* and controls the relocation of such genes to interchromatin granules, leading to the promotion of E2F1 sumoylation and activation of growth transcriptional programs (82).

11.2. Role of *MALAT1* in tumorigenesis

Several studies have shown that *MALAT1* plays a pivotal role in the malignancy phenotypes of cancer. Hence, *MALAT1* has been associated with cancer growth, invasion and metastasis (74, 175). *MALAT1* was originally identified as a lncRNA upregulated at early-stage in non-small cell lung cancers, which has a propensity for metastasis (162). Following studies have shown that *MALAT1* overexpression is related to multiple types of tumour, *e.g.* in the liver (176-180), breast (181-185) and colon (186-188). However, the target genes of *MALAT1* are variable among various types of cancer. Interestingly, chromosomal translocation breakpoints associated

with cancer have also been identified within the *MALAT1* locus (189-191).

MALAT1 can promote tumour development by other mechanisms that do not involve Polycomb. For instance, it has been reported that *MALAT1* binds competitively to the tumour suppressor gene splicing factor, poly-glutamine rich (SFPQ), thus releasing it from a SFPQ/PTBP2 complex (polypyrimidine-tract-binding protein), which leads to an increased level of PTBP2 alone (192). Upregulation of *MALAT1* promoted chemokine ligand 5 signalling, resulting in increased level of Snail and EMT promotion (193). Furthermore, *MALAT1* enhanced stemness in pancreatic cancer cells and in glioma stem cell lines by promoting the expression of self-renewal related factors through *Sox2* (194-195). In addition, *MALAT1* can activate the ERK/MAPK pathway, which orchestrates cancer progression by inducing transcriptional programs that regulate cell invasion (196). On the contrary, a recent study has shown that *MALAT1* functions as a tumour suppressor by inactivating ERK/MAPK signalling pathway in glioma cells (197). Further studies will be crucial in order to elucidate whether these discrepancies are due to the usage of different cell lines. Nonetheless, depletion of *MALAT1* has been associated with induced apoptosis leading to a decreased proliferation and suppression of the tumorigenic phenotype in many different cancer cell lines (198-201). Furthermore, *MALAT1* levels are regulated during cell division, influencing the expression of genes involved in cell cycle progression. In addition, depletion of *MALAT1* results in induction of the p53-mediated DNA damage response pathway in normal human diploid fibroblasts (202).

12. *HOTAIR*

HOTAIR is a spliced and polyadenylated lncRNA of 2,158 nt (76). This lncRNA is transcribed from the antisense strand of the *HOXC* locus located on the Chromosome 12. In mammals, 39 *HOX* encoding genes are grouped into four clusters (*HOXA-D*) on different chromosomes. During development, *HOX* expression occurs in a precise temporal and spatial sequence, which follows their chromosomal order (203).

HOTAIR has been proposed to silence *HOXD* genes *in trans* by recruiting PRC2 (76). Deletion of *HOTAIR* in human fibroblasts induced the expression of different members of the *HOX* family, which was associated with a loss of the H3K27me3 mark on the *HOXD* locus (76). However, it was thought initially that the function of *HOTAIR* was specific to human since mice bearing a complete deletion of the *HoxC* locus exhibited no significant changes in *HoxD* expression or chromatin marks (204). Nonetheless, a later study showed that deletion of mouse *Hotair* resulted in the derepression of *HoxD* genes and several imprinted loci (205). This study suggested that the lack of effect on the previously reported model was due to the concomitant deletion of all *HoxC* genes, which may have masked or compensated a potential alteration caused by the absence of *Hotair* alone (204). To date, four different alleles that result in either a partial or a complete deletion of *Hotair*, have been reported without reaching a consensus on the function of this lncRNA (204-208). Such differences could be explained by the use of different genetic background and their influence on knockout phenotypes (209).

12.1. *HOTAIR* and Polycomb

HOTAIR adopts a modular secondary structure at its 5' end, which is critical for PRC2 binding *in vitro* (210, 211). *HOTAIR* also contains a 3' domain that interacts with LSD1/CoREST/REST that catalyses H3K4me2 demethylation, serving as a scaffold for distinct histone modification complexes. The complex then targets the *HOXD* locus on chromosome 2 to silence genes involved in the suppression of metastasis (211). *HOTAIR* was previously thought to localize and target PRC2 genome-wide to modulate the cancer epigenome (212), but a recent study has shown that *HOTAIR* can also repress transcription independently of PRC2 by interacting with as yet unknown factors (213).

A *HOTAIR*-PRC2 interaction *in vivo* may occur by intermediate bridging proteins such as JARID2 (77). Furthermore, it has been shown that phosphorylation of EZH2 at threonine 345 increases its binding affinity for *HOTAIR* (46). Another study has shown that *HOTAIR* overexpression can induce the interaction of a complex containing SUZ12 and LSD1 with target genes, while depletion of *HOTAIR* has the opposite effect (211).

12.2. Role of *HOTAIR* in tumorigenesis

Aberrant expression of *HOTAIR* has been reported in various cancers, including breast, colorectal, pancreas or lung (214). Hence, *HOTAIR* could be used as a prognostic biomarker and as a predictor of patient survival in numerous cancer types (214). Furthermore, *HOTAIR* overexpression has been associated with metastasis (215). Nevertheless, the underlying mechanisms by which *HOTAIR* is involved in cancer development and metastasis remain poorly understood.

In breast cancer cells in which *HOTAIR* is overexpressed, SUZ12 and EZH2 are actively recruited to the H3K27me3 signature in the promoters of 854 genes (212). Most of these genes encode for tumour suppressor proteins such as progesterone receptor, *HOXD10* and protocadherin, suggesting that *HOTAIR* facilitates PRC2 occupancy in order to downregulate their expression. Consistently, depletion of EZH2 or SUZ12 induced gene expression, confirming the interplay between *HOTAIR* and PRC2 to regulate

transcription. *In vivo*, an intact PRC2 complex was required in order to induce breast cancer invasiveness by *HOTAIR* (212).

HOTAIR has been shown to regulate several other processes associated with carcinogenesis, such as EMT and acquisition of stemness (216), independently of Polycomb. For instance, epithelial ovarian cancer was associated with high level of *HOTAIR*, reduced survival and lymph node metastasis. On the contrary, suppression of *HOTAIR* reduced cell invasiveness. This pro-metastatic effect of *HOTAIR* has been shown to be mediated by matrix metalloproteinases (217). Another study has shown that *HOTAIR* promoter possesses numerous estrogen-response-elements that are targeted by E2 to regulate *HOTAIR* expression (218). Additionally, estrogen receptors (ERα and ERβ) and ER co-regulators also activated *HOTAIR* by binding to the promoter regions in presence of E2. *HOTAIR* expression has also been reported to be regulated by *c-Myc* (219). In gallbladder cancer tissues, *HOTAIR* lncRNA levels correlated positively with *c-Myc,* but negatively with miR-130a expression. Knockdown of *HOTAIR* inhibited gallbladder cancer cell invasiveness, whereas such cell invasiveness was rescued by miR-130a, suggesting that oncogenic *HOTAIR* activity occurs *via* the negative regulation of miRNA-130a (219). In the same way, it has been shown that in renal carcinoma cells, *HOTAIR* is involved in cell invasion whereas miR-141 suppressed *HOTAIR* oncogenic effects (220).

13. CONCLUDING REMARKS

Despite considerable development in our understanding of lncRNAs over the past decade, the functions of the majority of lncRNAs are unknown, and many lncRNAs may not have been discovered yet. Only a fraction of annotated lncRNAs has been examined for biological function revealing that the alteration of lncRNA homeostasis can adversely affect cellular identity, thereby promoting cancer development in a tissue-specific and/or cell-type-specific fashion. As discussed in the previous sections, lncRNAs can have both tumour suppressive and oncogenic roles. In addition, they can be regulated by multiple mechanisms, such as chemical modification, increasing the complexity of the number of possible levels of lncRNA regulation. Although the roles of several classically defined lncRNA and their associated PcGs have been discussed in this review, many potential functions are still unexplained or remain controversial. As Polycomb in mammals lack clear DNA sequence specificity, an exciting general model in whereby lncRNAs recruited PcGs, specifically PRC2, to chromatin emerged. But since PRC2 binding to RNA is promiscuous, what is the relevance of lncRNA-Polycomb interaction? Yet, the biological function of this interaction remains unknown. Furthermore, how lncRNA can guide PcGs to specific targets is not fully elucidated mechanistically. Future studies should aim to answer these and other open questions, as well as to define the functions of each lncRNA systematically, in order to reveal the molecular mechanisms underlying tumorigenesis in different cancer types.

RNA-based therapeutics, such as antisense oligonucleotides (ASOs) or siRNAs aimed at targeting an lncRNA of interest for degradation, or the use of small molecules to disrupt lncRNAs interactions, will lead to new strategies for cancer therapy. However, despite growing knowledge about the function of lncRNAs in cancer, a broader understanding of the molecular mechanism of action, and the regulatory pathways, hierarchies and networks in which lncRNA and associated chromatin complexes operate, is the essential first step for therapeutic development. We predict that such knowledge will aid in unlocking the full potential of this intricate network of molecules, and will facilitate the prediction of cancer risk, track the prognosis of tumour fate and provide novel therapeutic approaches.

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15. REFERENCES

1. Littauer, U.Z., and Inouye, H. Regulation of tRNA. *Annu Rev Biochem* 42, 439-470 (1973)
DOI: 10.1146/annurev.bi.42.070173.002255

2. Scherrer, K., Latham, H., and Darnell, J.E. Demonstration of an unstable RNA and of a precursor to ribosomal RNA in HeLa cells. *Proc Natl Acad Sci U S A* 49, 240-248 (1963)
DOI: 10.1073/pnas.49.2.240

3. Yang, V.W., Lerner, M.R., Steitz, J.A., and Flint, S.J. A Small Nuclear Ribonucleoprotein Is Required for Splicing of Adenoviral Early RNA Sequences. *Proc Natl Acad Sci USA* 78, 1371-1375 (1981)
DOI: 10.1073/pnas.78.3.1371

4. Maxwell, E.S., and Fournier, M.J. The Small Nucleolar RNAs. *Annu Rev of Biochem* 64,

897-934 (1995)
DOI: 10.1146/annurev.bi.64.070195.004341

5. Amaral, P.P., Dinger, M.E., and Mattick, J.S. Non-coding RNAs in homeostasis, disease and stress responses: an evolutionary perspective. *Briefings in Functional Genomics* 12, 254-278 (2013)
DOI: 10.1093/bfgp/elt016

6. Rupaimoole, R., and Slack, F.J. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov* 16, 203-222 (2017)
DOI: 10.1038/nrd.2016.246

7. Sen, R., Doose, G., and Stadler, P. Rare Splice Variants in Long Non-Coding RNAs. *Non-Coding. RNA* 3 (2017)

8. Dinger, M.E., Amaral, P.P., Mercer, T.R., Pang, K.C., Bruce, S.J., Gardiner, B.B., Askarian-Amiri, M.E., Ru, K., Solda, G., Simons, C., Sunkin, S.M., Crowe, M.L, Grimmond, S.M., Perkins, A.C., Mattick, J.S. Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. *Genome Res* 18, 1433-1445 (2008)
DOI: 10.1101/gr.078378.108

9. Flynn, R.A., and Chang, H.Y. Long Noncoding RNAs in Cell-Fate Programming and Reprogramming. *Cell Stem Cell* 14, 752-761 (2014)
DOI: 10.1016/j.stem.2014.05.014

10. Guttman, M., Russell, P., Ingolia, N.T., Weissman, J.S., and Lander, E.S. Ribosome profiling provides evidence that large noncoding RNAs do not encode proteins. *Cell* 154, 240-251 (2013)
DOI: 10.1016/j.cell.2013.06.009

11. Ruiz-Orera, J., Messeguer, X., Subirana, J.A., and Alba, M.M. Long non-coding RNAs as a source of new peptides. *Elife* 3 e03523 (2014)
DOI: 10.7554/eLife.03523

12. Carlevaro-Fita, J., Rahim, A., Guigo, R., Vardy, L.A., and Johnson, R. Cytoplasmic long noncoding RNAs are frequently bound to and degraded at ribosomes in human cells. *RNA* 22, 867-882 (2016)
DOI: 10.1261/rna.053561.115

13. Yoon, J.H., and Gorospe, M. Cross-Linking Immunoprecipitation and qPCR (CLIP-qPCR) Analysis to Map Interactions Between Long Noncoding RNAs and RNA-Binding Proteins. *Methods Mol Biol* 1402, 11-17 (2016)
DOI: 10.1007/978-1-4939-3378-5_2

14. Simon, M.D., Wang, C.I., Kharchenko, P.V., West, J.A., Chapman, B.A., Alekseyenko, A.A., Borowsky, M.L., Kuroda, M.I., and Kingston, R.E. The genomic binding sites of a noncoding RNA. *Proc Natl Acad Sci U S A* 108, 20497-20502 (2011)
DOI: 10.1073/pnas.1113536108

15. Chu, C., Quinn, J., and Chang, H.Y. Chromatin isolation by RNA purification (ChIRP). *J Vis Exp* (2012)
DOI: 10.3791/3912

16. Chu, C., Zhang, Q.C., da Rocha, S.T., Flynn, R.A., Bharadwaj, M., Calabrese, J.M., Magnuson, T., Heard, E., and Chang, H.Y. Systematic discovery of Xist RNA binding proteins. *Cell* 161, 404-416 (2015)
DOI: 10.1016/j.cell.2015.03.025

17. McHugh, C.A., Chen, C.K., Chow, A., Surka, C.F., Tran, C., McDonel, P., Pandya-Jones, A., Blanco, M., Burghard, C., Moradian, A., Sweredoski, M.J., Shishkin, A.A., Su, J., Lander, E.S., Hess, S., Plath, K., Guttman, M. The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3. *Nature* 521, 232-236 (2015)
DOI: 10.1038/nature14443

18. Khalil, A.M., Guttman, M., Huarte, M., Garber, M., Raj, A., Rivea Morales, D., Thomas, K., Presser, A., Bernstein, B.E., van Oudenaarden, A., Regev, A., Lander, E.S., Rinn, J.L. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc Natl Acad Sci U S A* 106, 11667-11672 (2009)
DOI: 10.1073/pnas.0904715106

19. Nakaya, H.I., Amaral, P.P., Louro, R., Lopes, A., Fachel, A.A., Moreira, Y.B., El-Jundi, T.A., da Silva, A.M., Reis, E.M., and Verjovski-Almeida, S. Genome mapping and expression analyses of human intronic noncoding RNAs reveal tissue-specific patterns and enrichment in genes related to regulation of transcription. *Genome Biol* 8, R43 (2007)
DOI: 10.1186/gb-2007-8-3-r43

20. Zhang, Y., Zhang, X.O., Chen, T., Xiang, J.F., Yin, Q.F., Xing, Y.H., Zhu, S., Yang, L.,

and Chen, L.L. Circular intronic long noncoding RNAs. *Mol Cell* 51, 792-806 (2013) DOI: 10.1016/j.molcel.2013.08.017

21. Lu, W., Han, L., Su, L., Zhao, J., Zhang, Y., Zhang, S., Zhao, B., and Miao, J. A 3'UTR-associated RNA, FLJ11812 maintains stemness of human embryonic stem cells by targeting miR-4459. *Stem Cells Dev* 24, 1133-1140 (2015) DOI: 10.1089/scd.2014.0353

22. Ulveling, D., Francastel, C., and Hube, F. When one is better than two: RNA with dual functions. *Biochimie* 93, 633-644 (2011) DOI: 10.1016/j.biochi.2010.11.004

23. Zhang, Y., Li, J.T., Kong, L., Gao, G., Liu, Q.R., and Wei, L.P. NATsDB: Natural antisense transcripts DataBase. *Nucleic Acids Res* 35, D156-D161 (2007) DOI: 10.1093/nar/gkl782

24. Aguilo, F., Li, S., Balasubramaniyan, N., Sancho, A., Benko, S., Zhang, F., Vashisht, A., Rengasamy, M., Andino, B., Chen, C.H., Zhou, F., Qian, C., Zhou, M.M., Wohlschlegel, J.A., Zhang, W., Suchy, F.J., Walsh, M.J. Deposition of 5-Methylcytosine on Enhancer RNAs Enables the Coactivator Function of PGC-1alpha. *Cell Rep* 14, 479-492 (2016) DOI: 10.1016/j.celrep.2015.12.043

25. Rothschild, G., and Basu, U. Lingering Questions about Enhancer RNA and Enhancer Transcription-Coupled Genomic Instability. *Trends Genet* 33, 143-154 (2017) DOI: 10.1016/j.tig.2016.12.002

26. Kim, T.K., Hemberg, M., Gray, J.M., Costa, A.M., Bear, D.M., Wu, J., Harmin, D.A., Laptewicz, M., Barbara-Haley, K., Kuersten, S., Markenscoff-Papadimitriou, E., Kuhl, D., Bito, H., Worley, P.F., Kreiman, G., and Greenberg, M.E. Widespread transcription at neuronal activity-regulated enhancers. *Nature* 465, 182-187 (2010) DOI: 10.1038/nature09033

27. Orom, U.A., Derrien, T., Beringer, M., Gumireddy, K., Gardini, A., Bussotti, G., Lai, F., Zytnicki, M., Notredame, C., Huang, Q., Guigo, R., and Shiekhattar, R. Long noncoding RNAs with enhancer-like function in human cells. *Cell* 143, 46-58 (2010) DOI: 10.1016/j.cell.2010.09.001

28. Pisignano, G., Napoli, S., Magistri, M., Mapelli, S.N., Pastori, C., Di Marco, S., Civenni, G., Albino, D., Enriquez, C., Allegrini, S., Mitra, A., D'Ambrosio, M., Mello-Grand, M., Chiorino, G., Garcia-Escudero, R., Varani, V., Carbone G.M., and Catapano, C.V. A promoter-proximal transcript targeted by genetic polymorphism controls E-cadherin silencing in human cancers. *Nat Commun* 8, 15622 (2017) DOI: 10.1038/ncomms15622

29. Schmitz, K.M., Mayer, C., Postepska, A., and Grummt, I. Interaction of noncoding RNA with the rDNA promoter mediates recruitment of DNMT3b and silencing of rRNA genes. *Genes Dev* 24, 2264-2269 (2010) DOI: 10.1101/gad.590910

30. Wang, X., Arai, S., Song, X., Reichart, D., Du, K., Pascual, G., Tempst, P., Rosenfeld, M.G., Glass, C.K., and Kurokawa, R. Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription. *Nature* 454, 126-130 (2008) DOI: 10.1038/nature06992

31. Preker, P., Almvig, K., Christensen, M.S., Valen, E., Mapendano, C.K., Sandelin, A., and Jensen, T.H. PROMoter uPstream Transcripts share characteristics with mRNAs and are produced upstream of all three major types of mammalian promoters. *Nucleic Acids Res* 39, 7179-7193 (2011) DOI: 10.1093/nar/gkr370

32. Tian, D., Sun, S., and Lee, J.T. The long noncoding RNA, Jpx, is a molecular switch for X chromosome inactivation. *Cell* 143, 390-403 (2010) DOI: 10.1016/j.cell.2010.09.049

33. Hung, T., Wang, Y.L., Lin, M.F., Koegel, A.K., Kotake, Y., Grant, G.D., Horlings, H.M., Shah, N., Umbricht, C., Wang, P., Kong, B., Langerod, A., Borresen-Dale, A.L., Kim, S.K., van de Vijver, M., Sukumar, S., Whitfield, M.L., Kellis, M., Xiong, Y., Wong, D.J., Chang, H.Y. Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. *Nature Genetics* 43, 621-U196 (2011) DOI: 10.1038/ng.848

34. Kino, T., Hurt, D.E., Ichijo, T., Nader, N., and Chrousos, G.P. Noncoding RNA Gas5 Is a Growth Arrest- and Starvation-Associated Repressor of the Glucocorticoid Receptor. *Sci Signal* 3 (2010) DOI: 10.1126/scisignal.2000568

35. Huarte, M., Guttman, M., Feldser, D., Garber, M., Koziol, M.J., Kenzelmann-Broz, D., Khalil, A.M., Zuk, O., Amit, I., Rabani, M., Attardi, L.D., Regev, A., Lander, E.S., Jacks, T., Rinn, J.L. A Large Intergenic Noncoding RNA Induced by p53 Mediates Global Gene Repression in the p53 Response. *Cell* 142, 409-419 (2010)
DOI: 10.1016/j.cell.2010.06.040

36. Spitale, R.C., Tsai, M.C., and Chang, H.Y. RNA templating the epigenome Long noncoding RNAs as molecular scaffolds. *Epigenetics-Us* 6, 539-543 (2011)
DOI: 10.4161/epi.6.5.15221

37. Bosson, A.D., Zamudio, J.R., and Sharp, P.A. Endogenous miRNA and Target Concentrations Determine Susceptibility to Potential ceRNA Competition. *Mol Cell* 56, 347-359 (2014)
DOI: 10.1016/j.molcel.2014.09.018

38. Salmena, L., Poliseno, L., Tay, Y., Kats, L., and Pandolfi, P.P. A ceRNA Hypothesis: The Rosetta Stone of a Hidden RNA Language? *Cell* 146, 353-358 (2011)
DOI: 10.1016/j.cell.2011.07.014

39. Cai, X.Z., and Cullen, B.R. The imprinted H19 noncoding RNA is a primary microRNA precursor. *RNA* 13, 313-316 (2007)
DOI: 10.1261/rna.351707

40. Keniry, A., Oxley, D., Monnier, P., Kyba, M., Dandolo, L., Smits, G., and Reik, W. The H19 lincRNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. *Nat Cell Biol* 14, 659-665 (2012)
DOI: 10.1038/ncb2521

41. Shafik, A., Schumann, U., Evers, M., Sibbritt, T., and Preiss, T. The emerging epitranscriptomics of long noncoding RNAs. *Biochim Biophys Acta* 1859, 59-70 (2016)
DOI: 10.1016/j.bbagrm.2015.10.019

42. Liu, N., Parisien, M., Dai, Q., Zheng, G., He, C., and Pan, T. Probing N6-methyladenosine RNA modification status at single nucleotide resolution in mRNA and long noncoding RNA. *RNA* 19, 1848-1856 (2013)
DOI: 10.1261/rna.041178.113

43. Liu, N., Dai, Q., Zheng, G., He, C., Parisien, M., and Pan, T. N(6)-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions. *Nature* 518, 560-564 (2015)
DOI: 10.1038/nature14234

44. Liu, N., Zhou, K.I., Parisien, M., Dai, Q., Diatchenko, L., and Pan, T. N6-methyladenosine alters RNA structure to regulate binding of a low-complexity protein. *Nucleic Acids Res* 45, 6051-6063 (2017)
DOI: 10.1093/nar/gkx141

45. Patil, D.P., Chen, C.K., Pickering, B.F., Chow, A., Jackson, C., Guttman, M., and Jaffrey, S.R. m(6)A RNA methylation promotes XIST-mediated transcriptional repression. *Nature* 537, 369-373 (2016)
DOI: 10.1038/nature19342

46. Amort, T., Souliere, M.F., Wille, A., Jia, X.Y., Fiegl, H., Worle, H., Micura, R., and Lusser, A. Long non-coding RNAs as targets for cytosine methylation. *RNA Biol* 10, 1003-1009 (2013)
DOI: 10.4161/rna.24454

47. Li, X., Zhu, P., Ma, S., Song, J., Bai, J., Sun, F., and Yi, C. Chemical pulldown reveals dynamic pseudouridylation of the mammalian transcriptome. *Nat Chem Biol* 11, 592-597 (2015)
DOI: 10.1038/nchembio.1836

48. Lewis, E.B. A gene complex controlling segmentation in Drosophila. *Nature* 276, 565-570 (1978)
DOI: 10.1038/276565a0

49. Levine, S.S., Weiss, A., Erdjument-Bromage, H., Shao, Z., Tempst, P., and Kingston, R.E. The Core of the Polycomb Repressive Complex Is Compositionally and Functionally Conserved in Flies and Humans. *Mol Cell Biol* 22, 6070-6078 (2002)
DOI: 10.1128/MCB.22.17.6070-6078.2002

50. Scheuermann, J.C., de Ayala Alonso, A.G., Oktaba, K., Ly-Hartig, N., McGinty, R.K., Fraterman, S., Wilm, M., Muir, T.W., and Muller, J. Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. *Nature* 465, 243-247 (2010)
DOI: 10.1038/nature08966

51. Dillon, S.C., Zhang, X., Trievel, R.C., and Cheng, X. The SET-domain protein super family: protein lysine methyltransferases. *Genome Biol* 6, 227 (2005)
DOI: 10.1186/gb-2005-6-8-227

52. Margueron, R., Li, G., Sarma, K., Blais, A., Zavadil, J., Woodcock, C.L., Dynlacht, B.D., and Reinberg, D. Ezh1 and Ezh2 maintain repressive chromatin through different mechanisms. *Mol Cell* 32, 503-518 (2008) DOI: 10.1016/j.molcel.2008.11.004

53. Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B.D., Sun, Z.W., Schmid, M., Opravil, S., Mechtler, K., Ponting, C.P., Allis, C.D., Jenuwein, T. Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 406, 593-599 (2000) DOI: 10.1038/35020506

54. Shen, X., Liu, Y., Hsu, Y.J., Fujiwara, Y., Kim, J., Mao, X., Yuan, G.C., and Orkin, S.H. EZH1 mediates methylation on histone H3 lysine 27 and complements EZH2 in maintaining stem cell identity and executing pluripotency. Mol Cell 32, 491-502 (2008) DOI: 10.1016/j.molcel.2008.10.016

55. Wang, H., Wang, L., Erdjument-Bromage, H., Vidal, M., Tempst, P., Jones, R.S., and Zhang, Y. Role of histone H2A ubiquitination in Polycomb silencing. *Nature* 431, 873-878 (2004) DOI: 10.1038/nature02985

56. Schwartz, Y.B., and Pirrotta, V. A new world of Polycombs: unexpected partnerships and emerging functions. *Nat Rev Genet* 14, 853-864 (2013) DOI: 10.1038/nrg3603

57. Dellino, G.I., Schwartz, Y.B., Farkas, G., McCabe, D., Elgin, S.C., and Pirrotta, V. Polycomb silencing blocks transcription initiation. *Mol Cell* 13, 887-893 (2004) DOI: 10.1016/S1097-2765(04)00128-5

58. Zhou, W., Zhu, P., Wang, J., Pascual, G., Ohgi, K.A., Lozach, J., Glass, C.K., and Rosenfeld, M.G. Histone H2A monoubiquitination represses transcription by inhibiting RNA polymerase II transcriptional elongation. *Mol Cell* 29, 69-80 (2008) DOI: 10.1016/j.molcel.2007.11.002

59. Tavares, L., Dimitrova, E., Oxley, D., Webster, J., Poot, R., Demmers, J., Bezstarosti, K., Taylor, S., Ura, H., Koide, H., Wutz, A., Vidal, M., Elderkin, S., and Brockdorff, N. RYBP-PRC1 complexes mediate H2A ubiquitylation at polycomb target sites independently of PRC2 and H3K27me3. *Cell* 148, 664-678 (2012) DOI: 10.1016/j.cell.2011.12.029

60. Chamberlain, S.J., Yee, D., and Magnuson, T. Polycomb repressive complex 2 is dispensable for maintenance of embryonic stem cell pluripotency. *Stem Cells* 26, 1496-1505 (2008) DOI: 10.1634/stemcells.2008-0102

61. Pasini, D., Bracken, A.P., Hansen, J.B., Capillo, M., and Helin, K. The polycomb group protein Suz12 is required for embryonic stem cell differentiation. *Mol Cell Biol* 27, 3769-3779 (2007) DOI: 10.1128/MCB.01432-06

62. Akasaka, T., van Lohuizen, M., van der Lugt, N., Mizutani-Koseki, Y., Kanno, M., Taniguchi, M., Vidal, M., Alkema, M., Berns, A., and Koseki, H. Mice doubly deficient for the Polycomb Group genes Mel18 and Bmi1 reveal synergy and requirement for maintenance but not initiation of Hox gene expression. *Development* 128, 1587-1597 (2001)

63. Bel, S., Core, N., Djabali, M., Kieboom, K., Van der Lugt, N., Alkema, M.J., and Van Lohuizen, M. Genetic interactions and dosage effects of Polycomb group genes in mice. *Development* 125, 3543-3551 (1998)

64. Sneeringer, C.J., Scott, M.P., Kuntz, K.W., Knutson, S.K., Pollock, R.M., Richon, V.M., and Copeland, R.A. Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. *Proc Natl Acad Sci U S A* 107, 20980-20985 (2010) DOI: 10.1073/pnas.1012525107

65. van Haaften, G., Dalgliesh, G.L., Davies, H., Chen, L., Bignell, G., Greenman, C., Edkins, S., Hardy, C., O'Meara, S., Teague, J., Butler, A., Hinton, J., Latimer, C., Andrews, J., Barthorpe, S., Beare, D., Buck, G., Campbell, P.J., Cole, J., Forbes, S., Jia, M., Jones, D., Kok, C.Y., Leroy, C., Lin, M.L., McBride, D.J., Maddison, M., Maquire, S., McLay, K., Menzies, A., Mironenko, T., Mulderrig, L., Mudie, L., Pleasance, E., Shepherd, R., Smith, R., Stebbings, L., Stephens, P., Tang, G., Tarpey, P.S., Turner, R., Turrell, K., Varian, J., West, S., Widaa, S., Wray, P., Collins, V.P., Ichimura, K., Law S., Wong J., Yuen, S.T., Leung, S.Y., Tonon, G., DePinho, R.A., Tai, Y.T., Anderson, K.C., Kahnoski, R.J., Massie, A., Khoo, S.K., Teh, B.T., Stratton M.R., and Futreal P.A. Somatic mutations of the histone H3K27

demethylase gene UTX in human cancer. *Nat Genet* 41, 521-523 (2009)
DOI: 10.1038/ng.349

66. Bracken, A.P., Kleine-Kohlbrecher, D., Dietrich, N., Pasini, D., Gargiulo, G., Beekman, C., Theilgaard-Monch, K., Minucci, S., Porse, B.T., Marine, J.C., Hansen, K.H., and Helin, K. The Polycomb group proteins bind throughout the INK4A-ARF locus and are disassociated in senescent cells. *Genes Dev* 21, 525-530 (2007)
DOI: 10.1101/gad.415507

67. Kleer, C.G., Cao, Q., Varambally, S., Shen, R., Ota, I., Tomlins, S.A., Ghosh, D., Sewalt, R.G., Otte, A.P., Hayes, D.F., Sabel, M.S., Livant, D., Weiss, S.J., Rubin, and M.A., Chinnaiyan, A.M. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc Natl Acad Sci U S A* 100, 11606-11611 (2003)
DOI: 10.1073/pnas.1933744100

68. Varambally, S., Dhanasekaran, S.M., Zhou, M., Barrette, T.R., Kumar-Sinha, C., Sanda, M.G., Ghosh, D., Pienta, K.J., Sewalt, R.G.A.B., Otte, A.P., Rubin, M.A., and Chinnaiyan, A.M. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* 419, 624-629 (2002)
DOI: 10.1038/nature01075

69. Zingg, D., Debbache, J., Schaefer, S.M., Tuncer, E., Frommel, S.C., Cheng, P., Arenas-Ramirez, N., Haeusel, J., Zhang, Y., Bonalli, M., McCabe, M.T., Creasy, C.L., Levesque, M.P., Boyman, O., Santoro, R., Shakhova, O., Dummer, R., and Sommer, L. The epigenetic modifier EZH2 controls melanoma growth and metastasis through silencing of distinct tumour suppressors. *Nat Commun* 6 (2015)
DOI: 10.1038/ncomms7051

70. Mak, W., Baxter, J., Silva, J., Newall, A.E., Otte, A.P., and Brockdorff, N. Mitotically stable association of polycomb group proteins Eed and Enx1 with the inactive X chromosome in trophoblast stem cells. *Curr Biol* 12, 1016-1020 (2002)
DOI: 10.1016/S0960-9822(02)00892-8

71. Zhao, J., Sun, B.K., Erwin, J.A., Song, J.J., and Lee, J.T. Polycomb Proteins Targeted by a Short Repeat RNA to the Mouse X Chromosome. *Science* 322, 750-756 (2008)
DOI: 10.1126/science.1163045

72. Kotake, Y., Nakagawa, T., Kitagawa, K., Suzuki, S., Liu, N., Kitagawa, M., and Xiong, Y. Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15(INK4B) tumor suppressor gene. *Oncogene* 30, 1956-1962 (2011)
DOI: 10.1038/onc.2010.568

73. Yap, K.L., Li, S.D., Munoz-Cabello, A.M., Raguz, S., Zeng, L., Mujtaba, S., Gil, J., Walsh, M.J., and Zhou, M.M. Molecular Interplay of the Noncoding RNA ANRIL and Methylated Histone H3 Lysine 27 by Polycomb CBX7 in Transcriptional Silencing of INK4a. *Mol Cell* 38, 662-674 (2010)
DOI: 10.1016/j.molcel.2010.03.021

74. Gutschner, T., Hammerle, M., and Diederichs, S. MALAT1 -- a paradigm for long noncoding RNA function in cancer. *J Mol Med (Berl)* 91, 791-801 (2013)
DOI: 10.1007/s00109-013-1028-y

75. Wu, Y., Huang, C., Meng, X., and Li, J. Long Noncoding RNA MALAT1: Insights into its Biogenesis and Implications in Human Disease. *Curr Pharm Des* 21, 5017-5028 (2015)
DOI: 10.2174/1381612821666150724115625

76. Rinn, J.L., Kertesz, M., Wang, J.K., Squazzo, S.L., Xu, X., Brugmann, S.A., Goodnough, L.H., Helms, J.A., Farnham, P.J., Segal, E., and Chang, H.Y. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell* 129, 1311-1323 (2007)
DOI: 10.1016/j.cell.2007.05.022

77. Kaneko, S., Bonasio, R., Saldana-Meyer, R., Yoshida, T., Son, J., Nishino, K., Umezawa, A., and Reinberg, D. Interactions between JARID2 and noncoding RNAs regulate PRC2 recruitment to chromatin. *Mol Cell* 53, 290-300 (2014)
DOI: 10.1016/j.molcel.2013.11.012

78. Zhao, J., Ohsumi, T.K., Kung, J.T., Ogawa, Y., Grau, D.J., Sarma, K., Song, J.J., Kingston, R.E., Borowsky, M., and Lee, J.T. Genome-wide identification of polycomb-associated RNAs by RIP-seq. *Mol Cell* 40, 939-953 (2010)
DOI: 10.1016/j.molcel.2010.12.011

79. Davidovich, C., Zheng, L., Goodrich, K.J., and Cech, T.R. Promiscuous RNA binding by Polycomb repressive complex 2. *Nat Struct Mol Biol* 20, 1250-1257 (2013)
DOI: 10.1038/nsmb.2679

80. Beltran, M., Yates, C.M., Skalska, L., Dawson, M., Reis, F.P., Viiri, K., Fisher, C.L., Sibley, C.R., Foster, B.M., Bartke, T., Ule, J., and Jenner, R.G. The interaction of PRC2 with RNA or chromatin is mutually antagonistic. *Genome Res* 26, 896-907 (2016) DOI: 10.1101/gr.197632.115

81. Yap, K.L., Li, S., Munoz-Cabello, A.M., Raguz, S., Zeng, L., Mujtaba, S., Gil, J., Walsh, M.J., and Zhou, M.M. Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. *Mol Cell* 38, 662-674 (2010) DOI: 10.1016/j.molcel.2010.03.021

82. Yang, L., Lin, C., Liu, W., Zhang, J., Ohgi, K.A., Grinstein, J.D., Dorrestein, P.C., and Rosenfeld, M.G. ncRNA- and Pc2 methylation-dependent gene relocation between nuclear structures mediates gene activation programs. *Cell* 147, 773-788 (2011) DOI: 10.1016/j.cell.2011.08.054

83. Brown, C.J., Hendrich, B.D., Rupert, J.L., Lafreniere, R.G., Xing, Y., Lawrence, J., and Willard, H.F. The human XIST gene: analysis of a 17 kb inactive X-specific RNA that contains conserved repeats and is highly localized within the nucleus. *Cell* 71, 527-542 (1992) DOI: 10.1016/0092-8674(92)90520-M

84. Penny, G.D., Kay, G.F., Sheardown, S.A., Rastan, S., and Brockdorff, N. Requirement for Xist in X chromosome inactivation. *Nature* 379, 131-137 (1996) DOI: 10.1038/379131a0

85. Sheardown, S.A., Duthie, S.M., Johnston, C.M., Newall, A.E., Formstone, E.J., Arkell, R.M., Nesterova, T.B., Alghisi, G.C., Rastan, S., and Brockdorff, N. Stabilization of Xist RNA mediates initiation of X chromosome inactivation. *Cell* 91, 99-107 (1997) DOI: 10.1016/S0092-8674(01)80012-X

86. Barr, M.L., and Bertram E.G. A morphological distinction between neurones of the male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. *Nature* 163, 676 (1949) DOI: 10.1038/163676a0

87. Gendrel, A.V., and Heard, E. Fifty years of X-inactivation research. *Development* 138, 5049-5055 (2011) DOI: 10.1242/dev.068320

88. Lyon, M.F. Gene action in the X-chromosome of the mouse (Mus musculus L.). *Nature* 190, 372-373 (1961) DOI: 10.1038/190372a0

89. Huynh, K.D., and Lee, J.T. Inheritance of a pre-inactivated paternal X chromosome in early mouse embryos. *Nature* 426, 857-862 (2003) DOI: 10.1038/nature02222

90. Okamoto, I., Otte, A.P., Allis, C.D., Reinberg, D., and Heard, E. Epigenetic dynamics of imprinted X inactivation during early mouse development. *Science* 303, 644-649 (2004) DOI: 10.1126/science.1092727

91. Brockdorff, N., Ashworth, A., Kay, G.F., Cooper, P., Smith, S., McCabe, V.M., Norris, D.P., Penny, G.D., Patel, D., and Rastan, S. Conservation of position and exclusive expression of mouse Xist from the inactive X chromosome. *Nature* 351, 329-331 (1991) DOI: 10.1038/351329a0

92. Brown, C.J., Ballabio, A., Rupert, J.L., Lafreniere, R.G., Grompe, M., Tonlorenzi, R., and Willard, H.F. A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. *Nature* 349, 38-44 (1991) DOI: 10.1038/349038a0

93. Navarro, P., Pichard, S., Ciaudo, C., Avner, P., and Rougeulle, C. Tsix transcription across the Xist gene alters chromatin conformation without affecting Xist transcription: implications for X-chromosome inactivation. *Genes Dev* 19, 1474-1484 (2005) DOI: 10.1101/gad.341105

94. Sado, T., Hoki, Y., and Sasaki, H. Tsix silences Xist through modification of chromatin structure. *Dev Cell* 9, 159-165 (2005) DOI: 10.1016/j.devcel.2005.05.015

95. Sun, B.K., Deaton, A.M., and Lee, J.T. A transient heterochromatic state in Xist preempts X inactivation choice without RNA stabilization. *Mol Cell* 21, 617-628 (2006) DOI:10.1016/j.molcel.2006.01.028

96. Ogawa, Y., Sun, B.K., and Lee, J.T. Intersection of the RNA interference and X-inactivation pathways. *Science* 320, 1336-1341 (2008) DOI: 10.1126/science.1157676

97. Payer, B., Rosenberg, M., Yamaji, M., Yabuta, Y., Koyanagi-Aoi, M., Hayashi, K., Yamanaka, S., Saitou, M., and Lee, J.T. Tsix RNA and the germline factor, PRDM14, link X reactivation and stem cell reprogramming. *Mol Cell* 52, 805-818 (2013)
DOI: 10.1016/j.molcel.2013.10.023

98. Ogawa, Y., and Lee, J.T. Xite, X-inactivation intergenic transcription elements that regulate the probability of choice. *Mol Cell* 11, 731-743 (2003)
DOI: 10.1016/S1097-2765(03)00063-7

99. Tsai, C.L., Rowntree, R.K., Cohen, D.E., and Lee, J.T. Higher order chromatin structure at the X-inactivation center via looping DNA. *Dev Biol* 319, 416-425 (2008)
DOI: 10.1016/j.ydbio.2008.04.010

100. Brockdorff, N. Polycomb complexes in X chromosome inactivation. *Philos Trans R Soc Lond B Biol Sci* 372 (2017)
DOI: 10.1098/rstb.2017.0021

101. Kohlmaier, A., Savarese, F., Lachner, M., Martens, J., Jenuwein, T., and Wutz, A. A chromosomal memory triggered by Xist regulates histone methylation in X inactivation. *PLoS Biol* 2, E171 (2004)
DOI: 10.1371/journal.pbio.0020171

102. Plath, K., Talbot, D., Hamer, K.M., Otte, A.P., Yang, T.P., Jaenisch, R., and Panning, B. Developmentally regulated alterations in Polycomb repressive complex 1 proteins on the inactive X chromosome. *J Cell Biol* 167, 1025-1035 (2004)
DOI: 10.1083/jcb.200409026

103. Plath , K., Fang, J., Mlynarczyk-Evans, S.K., Cao, R., Worringer, K.A., Wang, H., de la Cruz, C.C., Otte, A.P., Panning, B., and Zhang, Y. Role of histone H3 lysine 27 methylation in X inactivation. *Science* 300, 131-135 (2003)
DOI: 10.1126/science.1084274

104. Silva, J., Mak, W., Zvetkova, I., Appanah, R., Nesterova, T.B., Webster, Z., Peters, A.H., Jenuwein, T., Otte, A.P., and Brockdorff, N. Establishment of histone h3 methylation on the inactive X chromosome requires transient recruitment of Eed-Enx1 polycomb group complexes. *Dev Cell* 4, 481-495 (2003)
DOI: 10.1016/S1534-5807(03)00068-6

105. Wutz, A., Rasmussen, T.P., and Jaenisch, R. Chromosomal silencing and localization are mediated by different domains of Xist RNA. *Nat Genet* 30, 167-174 (2002)
DOI: 10.1038/ng820

106. Kaneko, S., Li, G., Son, J., Xu, C.F., Margueron, R., Neubert, T.A., and Reinberg, D. Phosphorylation of the PRC2 component Ezh2 is cell cycle-regulated and up-regulates its binding to ncRNA. *Genes Dev* 24, 2615-2620 (2010)
DOI: 10.1101/gad.1983810

107. Kanhere, A., Viiri, K., Araujo, C.C., Rasaiyaah, J., Bouwman, R.D., Whyte, W.A., Pereira, C.F., Brookes, E., Walker, K., Bell, G.W., Pombo, A., Fisher, A.G., Young, R.A., Jenner, R.G. Short RNAs are transcribed from repressed polycomb target genes and interact with polycomb repressive complex-2. *Mol Cell* 38, 675-688 (2010)
DOI: 10.1016/j.molcel.2010.03.019

108. da Rocha, S.T., Boeva, V., Escamilla-Del-Arenal, M., Ancelin, K., Granier, C., Matias, N.R., Sanulli, S., Chow, J., Schulz, E., Picard, C., Kaneko, S., Helin, K., Reinberg, D., Stewart, A.F., Wutz, A., Margueron, R., Heard, E. Jarid2 Is Implicated in the Initial Xist-Induced Targeting of PRC2 to the Inactive X Chromosome. *Mol Cell* 53, 301-316 (2014)
DOI: 10.1016/j.molcel.2014.01.002

109. Pinter, S.F., Sadreyev, R.I., Yildirim, E., Jeon, Y., Ohsumi, T.K., Borowsky, M., and Lee, J.T. Spreading of X chromosome inactivation via a hierarchy of defined Polycomb stations. *Genome Res* 22, 1864-1876 (2012)
DOI: 10.1101/gr.133751.111

110. Mak, W., Nesterova, T.B., de Napoles, M., Appanah, R., Yamanaka, S., Otte, A.P., and Brockdorff, N. Reactivation of the paternal X chromosome in early mouse embryos. *Science* 303, 666-669 (2004)
DOI: 10.1126/science.1092674

111. Cerase, A., Smeets, D., Tang, Y.A., Gdula, M., Kraus, F., Spivakov, M., Moindrot, B., Leleu, M., Tattermusch, A., Demmerle, J., Nesterova, T.B., Green, C., Otte, A.P., Schermelleh, L., Brockdorff, N. Spatial separation of Xist RNA and polycomb proteins revealed by superresolution microscopy. *Proc Natl Acad Sci U S A* 111, 2235-2240 (2014)
DOI: 10.1073/pnas.1312951111

112. de Napoles, M., Mermoud, J.E., Wakao, R., Tang, Y.A., Endoh, M., Appanah, R.,

Nesterova, T.B., Silva, J., Otte, A.P., Vidal, M., Koseki, H., Brockdorff, N. Polycomb group proteins Ring1A/B link ubiquitylation of histone H2A to heritable gene silencing and X inactivation. *Dev Cell* 7, 663-676 (2004) DOI: 10.1016/j.devcel.2004.10.005

113. Almeida, M., Pintacuda, G., Masui, O., Koseki, Y., Gdula, M., Cerase, A., Brown, D., Mould, A., Innocent, C., Nakayama, M., Schermelleh, L., Nesterova, T.B., Koseki, H., and Brockdorff, N. PCGF3/5-PRC1 initiates Polycomb recruitment in X chromosome inactivation. *Science* 356, 1081-1084 (2017) DOI: 10.1126/science.aal2512

114. Schoeftner, S., Sengupta, A.K., Kubicek, S., Mechtler, K., Spahn, L., Koseki, H., Jenuwein, T., and Wutz, A. Recruitment of PRC1 function at the initiation of X inactivation independent of PRC2 and silencing. *EMBO J* 25, 3110-3122 (2006) DOI: 10.1038/sj.emboj.7601187

115. Leeb, M., and Wutz, A. Ring1B is crucial for the regulation of developmental control genes and PRC1 proteins but not X inactivation in embryonic cells. *J Cell Biol* 178, 219-229 (2007) DOI: 10.1083/jcb.200612127

116. Kimel, V.M. Clinical-cytological correlations of mammary carcinoma based upon sex-chromatin counts; a preliminary study. *Cancer* 10, 922-927 (1957) DOI:/10.1002/1097-0142(195709/10)10:5<922::AID-CNCR2820 100509>3.0.CO;2-7

117. Moore, K.L., and Barr, M.L. The sex chromatin in human malignant tissues. *Br J Cancer* 11, 384-390 (1957) DOI: 10.1038/bjc.1957.45

118. Perry, M. Evaluation of breast tumour sex chromatin (Barr body) as an index of survival and response to pituitary ablation. *Br J Surg* 59, 731-734 (1972) DOI: 10.1002/bjs.1800590912

119. Savino, A., and Koss, L.G. The evaluation of sex chromatin as a prognostic factor in carcinoma of the breast. A preliminary report. *Acta Cytol* 15, 372-374 (1971)

120. Camargo, M., and Wang, N. Cytogenetic evidence for the absence of an inactivated X chromosome in a human female (XX) breast carcinoma cell line. *Hum Genet* 55, 81-85 (1980) DOI: 10.1007/BF00329131

121. Dutrillaux, B., Muleris, M., and Seureau, M.G. Imbalance of sex chromosomes, with gain of early-replicating X, in human solid tumors. *Int J Cancer* 38, 475-479 (1986) DOI: 10.1002/ijc.2910380404

122. Wang, N., Cedrone, E., Skuse, G.R., Insel, R., and Dry, J. Two identical active X chromosomes in human mammary carcinoma cells. *Cancer Genet Cytogenet* 46, 271-280 (1990) DOI: 10.1016/0165-4608(90)90112-N

123. Teixeira, M.R., Pandis, N., Dietrich, C.U., Reed, W., Andersen, J., Qvist, H., and Heim, S. Chromosome banding analysis of gynecomastias and breast carcinomas in men. *Genes Chromosomes Cancer 23*, 16-20 (1998) DOI: 10.1002/(SICI)1098-2264(199809)23:1<16::AID-GCC3>3.0.CO;2-9

124. Wolman, S.R., Sanford, J., Ratner, S., and Dawson, P.J. Breast cancer in males: DNA content and sex chromosome constitution. *Mod Pathol* 8, 239-243 (1995)

125. Swerdlow, A.J., Hermon, C., Jacobs, P.A., Alberman, E., Beral, V., Daker, M., Fordyce, A., and Youings, S. Mortality and cancer incidence in persons with numerical sex chromosome abnormalities: a cohort study. *Ann Hum Genet* 65, 177-188 (2001) DOI: 10.1046/j.1469-1809.2001.6520177.x

126. Huang, Y.S., Chang, C.C., Lee, S.S., Jou, Y.S., and Shih, H.M. Xist reduction in breast cancer upregulates AKTphosphorylation via HDAC3-mediated repression of PHLPP1 expression. *Oncotarget* 7, 43256-43266 (2016)

127. Richardson, A.L., Wang, Z.C., De Nicolo, A., Lu, X., Brown, M., Miron, A., Liao, X., Iglehart, J.D., Livingston, D.M., and Ganesan, S. X chromosomal abnormalities in basal-like human breast cancer. *Cancer Cell* 9, 121-132 (2006) DOI: 10.1016/j.ccr.2006.01.013

128. Scully, R., and Livingston, D.M. In search of the tumour-suppressor functions of BRCA1 and BRCA2. *Nature* 408, 429-432 (2000) DOI: 10.1038/35044000

129. Ganesan, S., Silver, D.P., Greenberg, R.A., Avni, D., Drapkin, R., Miron, A., Mok, S.C., Randrianarison, V., Brodie, S., Salstrom, J., Rasmussen, T.P., Klimke, A., Marrese, C., Marahrens, Y., Deng, C.X., Feunteun, J., and Livingston, D.M. BRCA1 supports XIST RNA concentration on the inactive X chromosome. *Cell* 111, 393-405 (2002) DOI: 10.1016/S0092-8674(02)01052-8

130. Ganesan, S., Silver, D.P., Drapkin, R., Greenberg, R., Feunteun, J., and Livingston, D.M. Association of BRCA1 with the inactive X chromosome and XIST RNA. *Philos Trans R Soc Lond B Biol Sci* 359, 123-128 (2004) DOI: 10.1098/rstb.2003.1371

131. Pageau, G.J., Hall, L.L., and Lawrence, J.B. BRCA1 does not paint the inactive X to localize XIST RNA but may contribute to broad changes in cancer that impact XIST and Xi heterochromatin. *J Cell Biochem* 100, 835-850 (2007) DOI: 10.1002/jcb.21188

132. Sirchia, S.M., Ramoscelli, L., Grati, F.R., Barbera, F., Coradini, D., Rossella, F., Porta, G., Lesma, E., Ruggeri, A., Radice, P., Simoni, G., Miozzo, M. Loss of the inactive X chromosome and replication of the active X in BRCA1-defective and wild-type breast cancer cells. *Cancer Res* 65, 2139-2146 (2005) DOI: 10.1158/0008-5472.CAN-04-3465

133. Xiao, C., Sharp, J.A., Kawahara, M., Davalos, A.R., Difilippantonio, M.J., Hu, Y., Li, W., Cao, L., Buetow, K., Ried, T., Chadwick, B.P., Deng, C.X., and Panning, B. The XIST noncoding RNA functions independently of BRCA1 in X inactivation. *Cell* 128, 977-989 (2007) DOI: 10.1016/j.cell.2007.01.034

134. Hall, L.L., Byron, M., Pageau, G., and Lawrence, J.B. AURKB-mediated effects on chromatin regulate binding versus release of XIST RNA to the inactive chromosome. *J Cell Biol* 186, 491-507 (2009) DOI: 10.1083/jcb.200811143

135. Yildirim, E., Kirby, J.E., Brown, D.E., Mercier, F.E., Sadreyev, R.I., Scadden, D.T., and Lee, J.T. Xist RNA is a potent suppressor of hematologic cancer in mice. *Cell* 152, 727-742 (2013) DOI: 10.1016/j.cell.2013.01.034

136. Savarese, F., Flahndorfer, K., Jaenisch, R., Busslinger, M., and Wutz, A. Hematopoietic precursor cells transiently reestablish permissiveness for X inactivation. *Mol Cell Biol* 26, 7167-7177 (2006) DOI: 10.1128/MCB.00810-06

137. Agrelo, R., Souabni, A., Novatchkova, M., Haslinger, C., Leeb, M., Komnenovic, V., Kishimoto, H., Gresh, L., Kohwi-Shigematsu, T., Kenner, L., and Wutz, A. SATB1 defines the developmental context for gene silencing by Xist in lymphoma and embryonic cells. *Dev Cell* 16, 507-516 (2009) DOI: 10.1016/j.devcel.2009.03.006

138. W., Gius, D., Onyango, P., Muldoon-Jacobs, K., Karp, J., Feinberg, A.P., and Cui, H. Epigenetic silencing of tumour suppressor gene p15 by its antisense RNA. *Nature* 451, 202-206 (2008) DOI: 10.1038/nature06468

139. Zhang, X., Hamblin, M.H., and Yin, K.J. The long noncoding RNA Malat1: Its physiological and pathophysiological functions. *RNA Biol*, 1-10 (2017) DOI: 10.1080/15476286.2017.1358347

140. Gil, J., and Peters, G. Regulation of the INK4b-ARF-INK4a tumour suppressor locus: all for one or one for all. *Nat Rev Mol Cell Biol* 7, 667-677 (2006) DOI: 10.1038/nrm1987

141. Popov, N., and Gil, J. Epigenetic regulation of the INK4b-ARF-INK4a locus: in sickness and in health. *Epigenetics* 5, 685-690 (2010) DOI: 10.4161/epi.5.8.12996

142. Behrmann, I., Wallner, S., Komyod, W., Heinrich, P.C., Schuierer, M., Buettner, R., and Bosserhoff, A.K. Characterization of methylthioadenosin phosphorylase (MTAP) expression in malignant melanoma. *Am J Pathol* 163, 683-690 (2003) DOI: 10.1016/S0002-9440(10)63695-4

143. Schmid, M., Malicki, D., Nobori, T., Rosenbach, M.D., Campbell, K., Carson, D.A., and Carrera, C.J. Homozygous deletions of methylthioadenosine phosphorylase (MTAP) are more frequent than p16INK4A (CDKN2) homozygous deletions in primary non-small cell lung cancers (NSCLC). *Oncogene* 17, 2669-2675 (1998) DOI: 10.1038/sj.onc.1202205

144. Holdt, L.M., Hoffmann, S., Sass, K., Langenberger, D., Scholz, M., Krohn, K., Finstermeier, K., Stahringer, A., Wilfert, W.,

Beutner, F., Gielen, S., Schuler, G., Gäbel, G., Bergert, H., Bechmann, I., Stadler, P.F., Thiery, J., and Teupser, D. Alu elements in ANRIL non-coding RNA at chromosome 9p21 modulate atherogenic cell functions through trans-regulation of gene networks. *PLoS Genet* 9, e1003588 (2013) DOI: 10.1371/journal.pgen.1003588

145. Jarinova, O., Stewart, A.F., Roberts, R., Wells, G., Lau, P., Naing, T., Buerki, C., McLean, B.W., Cook, R.C., Parker, J.S., and McPherson, R. Functional analysis of the chromosome 9p21.3. coronary artery disease risk locus. *Arterioscler Thromb Vasc Biol* 29, 1671-1677 (2009) DOI: 10.1161/ATVBAHA.109.189522

146. Rodriguez, C., Borgel, J., Court, F., Cathala, G., Forne, T., and Piette, J. CTCF is a DNA methylation-sensitive positive regulator of the INK/ARF locus. *Biochem Biophys Res Commun* 392, 129-134 (2010) DOI: 10.1016/j.bbrc.2009.12.159

147. Sato, K., Nakagawa, H., Tajima, A., Yoshida, K., and Inoue, I. ANRIL is implicated in the regulation of nucleus and potential transcriptional target of E2F1. *Oncol Rep* 24, 701-707 (2010)

148. Burd, C.E., Jeck, W.R., Liu, Y., Sanoff, H.K., Wang, Z., and Sharpless, N.E. Expression of linear and novel circular forms of an INK4/ARF-associated non-coding RNA correlates with atherosclerosis risk. *PLoS Genet* 6, e1001233 (2010) DOI: 10.1371/journal.pgen.1001233

149. Folkersen, L., Kyriakou, T., Goel, A., Peden, J., Malarstig, A., Paulsson-Berne, G., Hamsten, A., Hugh, W., Franco-Cereceda, A., Gabrielsen, A., and Eriksson, P. Relationship between CAD risk genotype in the chromosome 9p21 locus and gene expression. Identification of eight new ANRIL splice variants. *PLoS One* 4, e7677 (2009) DOI: 10.1371/journal.pone.0007677

150. Holdt, L.M., Stahringer, A., Sass, K., Pichler, G., Kulak, N.A., Wilfert, W., Kohlmaier, A., Herbst, A., Northoff, B.H., Nicolaou, A., Gäbel, G., Beutner, F., Scholz, M., Thiery, J., Musunuru, K., Krohn, K., Mann, M., and Teupser, D. Circular non-coding RNA ANRIL modulates ribosomal RNA maturation and atherosclerosis in humans. *Nat Commun* 7, 12429 (2016) DOI: 10.1038/ncomms12429

151. Sarkar, D., Oghabian, A., Bodiyabadu, P.K., Joseph, W.R., Leung, E.Y., Finlay, G.J., Baguley, B.C., and Askarian-Amiri, M.E. Multiple Isoforms of ANRIL in Melanoma Cells: Structural Complexity Suggests Variations in Processing. *Int J Mol Sci* 18 (2017) DOI: 10.3390/ijms18071378

152. Pasmant, E., Laurendeau, I., Heron, D., Vidaud, M., Vidaud, D., and Bieche, I. Characterization of a germ-line deletion, including the entire INK4/ARF locus, in a melanoma-neural system tumor family: identification of ANRIL, an antisense noncoding RNA whose expression coclusters with ARF. *Cancer Res* 67, 3963-3969 (2007) DOI: 10.1158/0008-5472.CAN-06-2004

153. Iacobucci, I., Sazzini, M., Garagnani, P., Ferrari, A., Boattini, A., Lonetti, A., Papayannidis, C., Mantovani, V., Marasco, E., Ottaviani, E., Soverini, S., Girelli, D., Luiselli, D., Vignetti, M., Baccarani, M., and Martinelli, G. A polymorphism in the chromosome 9p21 ANRIL locus is associated to Philadelphia positive acute lymphoblastic leukemia. *Leuk Res* 35, 1052-1059 (2011) DOI: 10.1016/j.leukres.2011.02.020

154. Chen, D., Zhang, Z., Mao, C., Zhou, Y., Yu, L., Yin, Y., Wu, S., Mou, X., and Zhu, Y. ANRIL inhibits p15(INK4b) through the TGFbeta1 signaling pathway in human esophageal squamous cell carcinoma. *Cell Immunol* 289, 91-96 (2014) DOI: 10.1016/j.cellimm.2014.03.015

155. Meseure, D., Vacher, S., Alsibai, K.D., Nicolas, A., Chemlali, W., Caly, M., Lidereau, R., Pasmant, E., Callens, C., and Bieche, I. Expression of ANRIL-Polycomb Complexes-CDKN2A/B/ARF Genes in Breast Tumors: Identification of a Two-Gene (EZH2/CBX7) Signature with Independent Prognostic Value. *Mol Cancer Res* 14, 623-633 (2016) DOI: 10.1158/1541-7786.MCR-15-0418

156. Zhang, E.B., Kong, R., Yin, D.D., You, L.H., Sun, M., Han, L., Xu, T.P., Xia, R., Yang, J.S., De, W., and Chen, J.F. Long noncoding RNA ANRIL indicates a poor prognosis of gastric cancer and promotes tumor growth by epigenetically silencing of miR-99a/miR-449a. *Oncotarget* 5, 2276-2292 (2014) DOI: 10.18632/oncotarget.1902

157. Zhu, H., Li, X., Song, Y., Zhang, P., Xiao, Y., and Xing, Y. Long non-coding RNA ANRIL is up-regulated in bladder cancer and regulates bladder cancer cell proliferation and apoptosis through the intrinsic pathway. *Biochem Biophys Res Commun* 467, 223-228 (2015)
DOI: 10.1016/j.bbrc.2015.10.002

158. Aguilo, F., Di Cecilia, S., and Walsh, M.J. Long Non-coding RNA ANRIL and Polycomb in Human Cancers and Cardiovascular Disease. *Curr Top Microbiol Immunol* 394, 29-39 (2016)
DOI: 10.1007/82_2015_455

159. Wan, G., Mathur, R., Hu, X., Liu, Y., Zhang, X., Peng, G., and Lu, X. Long non-coding RNA ANRIL (CDKN2B-AS) is induced by the ATM-E2F1 signaling pathway. *Cell Signal* 25, 1086-1095 (2013)
DOI: 10.1016/j.cellsig.2013.02.006

160. Ma, J., Li, T., Han, X., and Yuan, H. Knockdown of LncRNA ANRIL suppresses cell proliferation, metastasis, and invasion via regulating miR-122-5p expression in hepatocellular carcinoma. *J Cancer Res Clin Oncol* (2017)

161. Zhao, J.J., Hao, S., Wang, L.L., Hu, C.Y., Zhang, S., Guo, L.J., Zhang, G., Gao, B., Jiang, Y., Tian, W.G., and Luo, D.L. Long non-coding RNA ANRIL promotes the invasion and metastasis of thyroid cancer cells through TGF-beta/Smad signaling pathway. *Oncotarget* 7, 57903-57918 (2016)

162. Ji, P., Diederichs, S., Wang, W., Boing, S., Metzger, R., Schneider, P.M., Tidow, N., Brandt, B., Buerger, H., Bulk, E., Thomas, M., Berdel, W.E., Serve, H., and Müller-Tidow, C. MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. *Oncogene* 22, 8031-8041 (2003)
DOI: 10.1038/sj.onc.1206928

163. Nakagawa, S., Ip, J.Y., Shioi, G., Tripathi, V., Zong, X., Hirose, T., and Prasanth, K.V. Malat1 is not an essential component of nuclear speckles in mice. *RNA* 18, 1487-1499 (2012)
DOI: 10.1261/rna.033217.112

164. Eissmann, M., Gutschner, T., Hammerle, M., Gunther, S., Caudron-Herger, M., Gross, M., Schirmacher, P., Rippe, K., Braun, T., Diederichs, S., and Zornig, M. Loss of the abundant nuclear non-coding RNA MALAT1 is compatible with life and development. *RNA Biol* 9, 1076-1087 (2012)
DOI: 10.4161/rna.21089

165. Zhang, B., Arun, G., Mao, Y.S., Lazar, Z., Hung, G., Bhattacharjee, G., Xiao, X., Booth, C.J., Wu, J., Zhang, C., Spector, D.L. The lncRNA Malat1 is dispensable for mouse development but its transcription plays a cis-regulatory role in the adult. *Cell Rep* 2, 111-123 (2012)
DOI: 10.1016/j.celrep.2012.06.003

166. Wilusz, J.E., Freier, S.M., and Spector, D.L. 3' end processing of a long nuclear-retained noncoding RNA yields a tRNA-like cytoplasmic RNA. *Cell* 135, 919-932 (2008)
DOI: 10.1016/j.cell.2008.10.012

167. Brown, J.A., Valenstein, M.L., Yario, T.A., Tycowski, K.T., and Steitz, J.A. Formation of triple-helical structures by the 3'-end sequences of MALAT1 and MENbeta noncoding RNAs. *Proc Natl Acad Sci U S A* 109, 19202-19207 (2012)
DOI: 10.1073/pnas.1217338109

168. Wilusz, J.E., JnBaptiste, C.K., Lu, L.Y., Kuhn, C.D., Joshua-Tor, L., and Sharp, P.A. A triple helix stabilizes the 3' ends of long noncoding RNAs that lack poly(A) tails. *Genes Dev* 26, 2392-2407 (2012)
DOI: 10.1101/gad.204438.112

169. Hutchinson, J.N., Ensminger, A.W., Clemson, C.M., Lynch, C.R., Lawrence, J.B., and Chess, A. A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains. *BMC Genomics* 8, 39 (2007)
DOI: 10.1186/1471-2164-8-39

170. Tripathi, V., Ellis, J.D., Shen, Z., Song, D.Y., Pan, Q., Watt, A.T., Freier, S.M., Bennett, C.F., Sharma, A., Bubulya, P.A., Blencowe, B.J., Prasanth, S.G., and Prasanth, K.V. The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. *Mol Cell* 39, 925-938 (2010)
DOI: 10.1016/j.molcel.2010.08.011

171. West, J.A., Davis, C.P., Sunwoo, H., Simon, M.D., Sadreyev, R.I., Wang, P.I., Tolstorukov, M.Y., and Kingston, R.E. The long noncoding RNAs NEAT1 and MALAT1 bind active chromatin sites. *Mol Cell* 55, 791-802 (2014)
DOI: 10.1016/j.molcel.2014.07.012

172. Kim, S.H., Kim, S.H., Yang, W.I., Kim, S.J., and Yoon, S.O. Association of the long non-coding RNA MALAT1 with the polycomb repressive complex pathway in T and NK cell lymphoma. *Oncotarget* 8, 31305-31317 (2017) DOI: 10.18632/oncotarget.15453

173. Hirata, H., Hinoda, Y., Shahryari, V., Deng, G., Nakajima, K., Tabatabai, Z.L., Ishii, N., and Dahiya, R. Long Noncoding RNA MALAT1 Promotes Aggressive Renal Cell Carcinoma through Ezh2 and Interacts with miR-205. *Cancer Res* 75, 1322-1331 (2015) DOI: 10.1158/0008-5472.CAN-14-2931

174. Fan, Y., Shen, B., Tan, M., Mu, X., Qin, Y., Zhang, F., and Liu, Y. TGF-beta-induced upregulation of malat1 promotes bladder cancer metastasis by associating with suz12. *Clin Cancer Res* 20, 1531-1541 (2014) DOI: 10.1158/1078-0432.CCR-13-1455

175. Huarte, M. The emerging role of lncRNAs in cancer. *Nat Med* 21, 1253-1261 (2015) DOI: 10.1038/nm.3981

176. Guerrieri, F. Long non-coding RNAs era in liver cancer. *World J Hepatol* 7, 1971-1973 (2015) DOI: 10.4254/wjh.v7.i16.1971

177. Lin, R., Maeda, S., Liu, C., Karin, M., and Edgington, T.S. A large noncoding RNA is a marker for murine hepatocellular carcinomas and a spectrum of human carcinomas. *Oncogene* 26, 851-858 (2007) DOI: 10.1038/sj.onc.1209846

178. Liu, W.T., Lu, X., Tang, G.H., Ren, J.J., Liao, W.J., Ge, P.L., and Huang, J.F. LncRNAs expression signatures of hepatocellular carcinoma revealed by microarray. *World J Gastroenterol* 20, 6314-6321 (2014) DOI: 10.3748/wjg.v20.i20.6314

179. Mohamadkhani, A. Long Noncoding RNAs in Interaction With RNA Binding Proteins in Hepatocellular Carcinoma. *Hepat Mon* 14, e18794 (2014) DOI: 10.5812/hepatmon.18794

180. Wu, M., Lin, Z., Li, X., Xin, X., An, J., Zheng, Q., Yang, Y., and Lu, D. HULC cooperates with MALAT1 to aggravate liver cancer stem cells growth through telomere repeat-binding factor 2. *Sci Rep* 6, 36045 (2016) DOI: 10.1038/srep36045

181. Ellis, M.J., Ding, L., Shen, D., Luo, J., Suman, V.J., Wallis, J.W., Van Tine, B.A., Hoog, J., Goiffon, R.J., Goldstein, T.C., Ng, S., Lin, L., Crowder, R., Snider, J., Ballman, K.,Weber, J., Chen, K., Koboldt, D.C., Kandoth, C., Schierding, W.S., McMichael, J.F., Miller, C.A., Lu, C., Harris, C.C., McLellan, M.D., Wendl, M.C., DeSchryver, K., Allred, D.C., Esserman, L., Unzeitig, G., Margenthaler, J., Babiera,G.V., Marcom, P.K., Guenther, J.M., Leitch, M., Hunt, K., Olson, J., Tao, Y., Maher, C.A., Fulton, L.J., Fulton, R.S., Harrison, M., Oberkfell,B., Du,F., Demeter, R., Vickery, T.L., Elhammali, A., Piwnica-Worms, H., McDonald, S., Watson, M., Dooling, D.J, Ota, D., Chang, L-W., Bose, R., Ley, T.J., Piwnica-Worms, D., Stuart, J.M.,Wilson, R.K., and Mardis, E.R. Whole-genome analysis informs breast cancer response to aromatase inhibition. *Nature* 486, 353-360 (2012) DOI: 10.1038/nature11143

182. Guffanti, A., Iacono, M., Pelucchi, P., Kim, N., Solda, G., Croft, L.J., Taft, R.J., Rizzi, E., Askarian-Amiri, M., Bonnal, R.J., Callari, M., Mignone, F., Pesole, G., Bertalot, G., Bernardi, L.R., Albertini, A., Lee, C., Mattick, J.S., Zucchi, I., and De Bellis, G. A transcriptional sketch of a primary human breast cancer by 454 deep sequencing. *BMC Genomics* 10, 163 (2009) DOI: 10.1186/1471-2164-10-163

183. Huang, N.S., Chi, Y.Y., Xue, J.Y., Liu, M.Y., Huang, S., Mo, M., Zhou, S.L., and Wu, J. Long non-coding RNA metastasis associated in lung adenocarcinoma transcript 1 (MALAT1) interacts with estrogen receptor and predicted poor survival in breast cancer. *Oncotarget* 7, 37957-37965 (2016) DOI: 10.18632/oncotarget.9364

184. Xu, S., Sui, S., Zhang, J., Bai, N., Shi, Q., Zhang, G., Gao, S., You, Z., Zhan, C., Liu, F., and Pang, D. Downregulation of long noncoding RNA MALAT1 induces epithelial-to-mesenchymal transition via the PI3K-AKT pathway in breast cancer. *Int J Clin Exp Pathol* 8, 4881-4891 (2015)

185. Zhao, Z., Chen, C., Liu, Y., and Wu, C. 17beta-Estradiol treatment inhibits breast cell proliferation, migration and invasion by decreasing MALAT-1 RNA level. *Biochem Biophys Res Commun* 445, 388-393 (2014) DOI: 10.1016/j.bbrc.2014.02.006

186. Xu, C., Yang, M., Tian, J., Wang, X., and Li, Z. MALAT-1: a long non-coding RNA and its important 3' end functional motif in colorectal cancer metastasis. *Int J Oncol* 39, 169-175 (2011)

187. Yang, M.H., Hu, Z.Y., Xu, C., Xie, L.Y., Wang, X.Y., Chen, S.Y., and Li, Z.G. MALAT1 promotes colorectal cancer cell proliferation/migration/invasion via PRKA kinase anchor protein 9. *Biochim Biophys Acta* 1852, 166-174 (2015)
DOI: 10.1016/j.bbadis.2014.11.013

188. Yang, Y., Junjie, P., Sanjun, C., and Ma, Y. Long non-coding RNAs in Colorectal Cancer: Progression and Future Directions. *J Cancer* 8, 3212-3225 (2017)
DOI: 10.7150/jca.19794

189. Davis, I.J., Hsi, B.L., Arroyo, J.D., Vargas, S.O., Yeh, Y.A., Motyckova, G., Valencia, P., Perez-Atayde, A.R., Argani, P., Ladanyi, M., Fletcher, J.A., and Fisher, D.E. Cloning of an Alpha-TFEB fusion in renal tumors harboring the t(6;11)(p21;q13) chromosome translocation. *Proc Natl Acad Sci U S A* 100, 6051-6056 (2003)
DOI: 10.1073/pnas.0931430100

190. Kuiper, R.P., Schepens, M., Thijssen, J., van Asseldonk, M., van den Berg, E., Bridge, J., Schuuring, E., Schoenmakers, E.F., and van Kessel, A.G. Upregulation of the transcription factor TFEB in t(6;11)(p21;q13)-positive renal cell carcinomas due to promoter substitution. *Hum Mol Genet* 12, 1661-1669 (2003)
DOI: 10.1093/hmg/ddg178

191. Rajaram, V., Knezevich, S., Bove, K.E., Perry, A., and Pfeifer, J.D. DNA sequence of the translocation breakpoints in undifferentiated embryonal sarcoma arising in mesenchymal hamartoma of the liver harboring the t(11;19)(q11;q13.4.) translocation. *Genes Chromosomes Cancer* 46, 508-513 (2007)
DOI: 10.1002/gcc.20437

192. Ji, Q., Zhang, L., Liu, X., Zhou, L., Wang, W., Han, Z., Sui, H., Tang, Y., Wang, Y., Liu, N., Ren, J., Hou, F., and Li, Q. Long non-coding RNA MALAT1 promotes tumour growth and metastasis in colorectal cancer through binding to SFPQ and releasing oncogene PTBP2 from SFPQ/PTBP2 complex. *Br J Cancer* 111, 736-748 (2014)
DOI: 10.1038/bjc.2014.383

193. Kan, J.Y., Wu, D.C., Yu, F.J., Wu, C.Y., Ho, Y.W., Chiu, Y.J., Jian, S.F., Hung, J.Y., Wang, J.Y., and Kuo, P.L. Chemokine (C-C Motif) Ligand 5 is Involved in Tumor-Associated Dendritic Cell-Mediated Colon Cancer Progression Through Non-Coding RNA MALAT-1. *J Cell Physiol* 230, 1883-1894 (2015)
DOI: 10.1002/jcp.24918

194. Han, Y., Zhou, L., Wu, T., Huang, Y., Cheng, Z., Li, X., Sun, T., Zhou, Y., and Du, Z. Downregulation of lncRNA-MALAT1 Affects Proliferation and the Expression of Stemness Markers in Glioma Stem Cell Line SHG139S. *Cell Mol Neurobiol* 36, 1097-1107 (2016)
DOI: 10.1007/s10571-015-0303-6

195. Jiao, F., Hu, H., Han, T., Yuan, C., Wang, L., Jin, Z., Guo, Z., and Wang, L. Long noncoding RNA MALAT-1 enhances stem cell-like phenotypes in pancreatic cancer cells. *Int J Mol Sci* 16, 6677-6693 (2015)
DOI: 10.3390/ijms16046677

196. Wu, X.S., Wang, X.A., Wu, W.G., Hu, Y.P., Li, M.L., Ding, Q., Weng, H., Shu, Y.J., Liu, T.Y., Jiang, L., Cao, Y., Bao, R.F., Mu, J.S., Tan, Z.J., Tao, F., and Liu, Y.B. MALAT1 promotes the proliferation and metastasis of gallbladder cancer cells by activating the ERK/MAPK pathway. *Cancer Biol Ther* 15, 806-814 (2014)
DOI: 10.4161/cbt.28584

197. Han, Y., Wu, Z., Wu, T., Huang, Y., Cheng, Z., Li, X., Sun, T., Xie, X., Zhou, Y., and Du, Z. Tumor-suppressive function of long noncoding RNA MALAT1 in glioma cells by downregulation of MMP2 and inactivation of ERK/MAPK signaling. *Cell Death Dis* 7, e2123 (2016)
DOI: 10.1038/cddis.2015.407

198. Huang, J.L., Liu, W., Tian, L.H., Chai, T.T., Liu, Y., Zhang, F., Fu, H.Y., Zhou, H.R., and Shen, J.Z. Upregulation of long non-coding RNA MALAT-1 confers poor prognosis and influences cell proliferation and apoptosis in acute monocytic leukemia. *Oncol Rep* 38, 1353-1362 (2017)
DOI: 10.3892/or.2017.5802

199. Li, Z., Xu, C., Ding, B., Gao, M., Wei, X., and Ji, N. Long non-coding RNA MALAT1 promotes proliferation and suppresses apoptosis of glioma cells through derepressing Rap1B by sponging miR-101.

J Neurooncol 134, 19-28 (2017)
DOI: 10.1007/s11060-017-2498-5

200. Liu, S., Jiang, X., Li, W., Cao, D., Shen, K., and Yang, J. Inhibition of the long non-coding RNA MALAT1 suppresses tumorigenicity and induces apoptosis in the human ovarian cancer SKOV3 cell line. *Oncol Lett* 11, 3686-3692 (2016)
DOI: 10.3892/ol.2016.4435

201. Xie, H., Liao, X., Chen, Z., Fang, Y., He, A., Zhong, Y., Gao, Q., Xiao, H., Li, J., Huang, W., and Liu, Y. LncRNA MALAT1 Inhibits Apoptosis and Promotes Invasion by Antagonizing miR-125b in Bladder Cancer Cells. *J Cancer* 8, 3803-3811 (2017)
DOI: 10.7150/jca.21228

202. Tripathi, V., Shen, Z., Chakraborty, A., Giri, S., Freier, S.M., Wu, X., Zhang, Y., Gorospe, M., Prasanth, S.G., Lal, A., and Prasanth, K.V. Long noncoding RNA MALAT1 controls cell cycle progression by regulating the expression of oncogenic transcription factor B-MYB. *PLoS Genet* 9, e1003368 (2013)
DOI: 10.1371/journal.pgen.1003368

203. Mallo, M., and Alonso, C.R. The regulation of Hox gene expression during animal development. *Development* 140, 3951-3963 (2013)
DOI: 10.1242/dev.068346

204. Schorderet, P., and Duboule, D. Structural and functio*nal differe*nces in the long non-coding RNA hotair in mouse and human. PLoS Genet 7, e1002071 (2011)
DOI: 10.1371/journal.pgen.1002071

205. Li, L., Liu, B., Wapinski, O.L., Tsai, M.C., Qu, K., Zhang, J., Carlson, J.C., Lin, M., Fang, F., Gupta, R.A., Helms J.A., Chang, H.Y. Targeted disruption of Hotair leads to homeotic transformation and gene derepression. Cell Rep 5, 3-12 (2013)
DOI: 10.1016/j.celrep.2013.09.003

206. *Amandio, A.R., Necsulea, A., Joye, E., Mascrez, B., and Duboule, D. Hotair Is Dispensible for Mouse Development. PLoS Genet* 12, e1006232 (2016)
DOI: 10.1371/journal.pgen.1006232

207. Lai, K.M., Gong, G., Atanasio, A., Rojas, J., Quispe, J., Posca, J., White, D., Huang, M., Fedorova, D., Grant, C., Miloscio, L., Droguett, G., Poueymirou, W.T., Auerbach, W., Yancopoulos, G.D., Frendewey, D., Rinn, J., and Valenzuela, D.M. Diverse Phenotypes and Specific Transcription Patterns in Twenty Mouse Lines with Ablated LincRNAs. *PLoS One* 10, e0125522 (2015)
DOI: 10.1371/journal.pone.0125522

208. Selleri, L., Bartolomei, M.S., Bickmore, W.A., He, L., Stubbs, L., Reik, W., and Barsh, G.S. A Hox-Embedded Long Noncoding RNA: Is It All Hot Air? *PLoS Genet* 12, e1006485 (2016)
DOI: 10.1371/journal.pgen.1006485

209. Li, L., Helms, J.A., and Chang, H.Y. Comment on “Hotair Is Dispensable for Mouse Development”. *PLoS Genet* 12, e1006406 (2016)
DOI: 10.1371/journal.pgen.1006406

210. Somarowthu, S., Legiewicz, M., Chillon, I., Marcia, M., Liu, F., and Pyle, A.M. HOTAIR forms an intricate and modular secondary structure. *Mol Cell* 58, 353-361 (2015)
DOI: 10.1016/j.molcel.2015.03.006

211. Tsai, M.C., Manor, O., Wan, Y., Mosammaparast, N., Wang, J.K., Lan, F., Shi, Y., Segal, E., and Chang, H.Y. Long noncoding RNA as modular scaffold of histone modification complexes. *Science* 329, 689-693 (2010)
DOI: 10.1126/science.1192002

212. Gupta, R.A., Shah, N., Wang, K.C., Kim, J., Horlings, H.M., Wong, D.J., Tsai, M.C., Hung, T., Argani, P., Rinn, J.L., Wang, Y., Brzoska, P., Kong, B., Li, R., West, R.B., van de Vijver, M.J., Sukumar, S., and Chang, H.Y. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature* 464, 1071-1076 (2010)
DOI: 10.1038/nature08975

213. Portoso, M., Ragazzini, R., Brencic, Z., Moiani, A., Michaud, A., Vassilev, I., Wassef, M., Servant, N., Sargueil, B., and Margueron, R. PRC2 is dispensable for HOTAIR-mediated transcriptional repression. *Embo* J 36, 981-994 (2017)
DOI: 10.15252/embj.201695335

214. Hajjari, M., and Salavaty, A. HOTAIR: an oncogenic long non-coding RNA in different cancers. *Cancer Biol Med* 12, 1-9 (2015)

215. Heubach, J., Monsior, J., Deenen, R., Niegisch, G., Szarvas, T., Niedworok, C., Schulz, W.A., and Hoffmann, M.J. The long noncoding RNA HOTAIR has tissue and cell type-dependent effects on HOX gene expression and phenotype of urothelial cancer cells. *Mol Cancer* 14 (2015) DOI: 10.1186/s12943-015-0371-8

216. Padua Alves, C., Fonseca, A.S., Muys, B.R., de Barros, E.L.B.R., Burger, M.C., de Souza, J.E., Valente, V., Zago, M.A., and Silva, W.A., Jr. Brief report: The lincRNA Hotair is required for epithelial-to-mesenchymal transition and stemness maintenance of cancer cell lines. *Stem Cells* 31, 2827-2832. (2013) DOI: 10.1002/stem.1547

217. Qiu, J.J., Lin, Y.Y., Ye, L.C., Ding, J.X., Feng, W.W., Jin, H.Y., Zhang, Y., Li, Q., and Hua, K.Q. Overexpression of long non-coding RNA HOTAIR predicts poor patient prognosis and promotes tumor metastasis in epithelial ovarian cancer. *Gynecol Oncol* 134, 121-128 (2014) DOI: 10.1016/j.ygyno.2014.03.556

218. Bhan, A., Hussain, I., Ansari, K.I., Kasiri, S., Bashyal, A., and Mandal, S.S. Antisense transcript long noncoding RNA (lncRNA) HOTAIR is transcriptionally induced by estradiol. *J Mol Biol* 425, 3707-3722 (2013) DOI: 10.1016/j.jmb.2013.01.022

219. Ma, M.Z., Li, C.X., Zhang, Y., Weng, M.Z., Zhang, M.D., Qin, Y.Y., Gong, W., and Quan, Z.W. Long non-coding RNA HOTAIR, a c-Myc activated driver of malignancy, negatively regulates miRNA-130a in gallbladder cancer. *Mol Cancer* 13, 156 (2014) DOI: 10.1186/1476-4598-13-156

220. Chiyomaru, T., Fukuhara, S., Saini, S., Majid, S., Deng, G.R., Shahryari, V., Chang, I., Tanaka, Y., Enokida, H., Nakagawa, M., Dahiya, R., and Yamamura, S. Long Non-coding RNA HOTAIR Is Targeted and Regulated by miR-141 in Human Cancer Cells. *J Biol Chem* 289, 12550-12565 (2014) DOI: 10.1074/jbc.M113.488593

221. Chooniedass-Kothari, S., Emberley, E., Hamedani, M.K., Troup, S., Wang, X., Czosnek, A., Hube, F., Mutawe, M., Watson, P.H., and Leygue, E. The steroid receptor RNA activator is the first functional RNA encoding a protein. *Febs Letters* 566, 43-47 (2004) DOI: 10.1016/j.febslet.2004.03.104

222. Vanhee-Brossollet, C., and Vaquero, C. Do natural antisense transcripts make sense in eukaryotes? *Gene* 211, 1-9 (1998) DOI: 10.1016/S0378-1119(98)00093-6

223. Taft, R.J., Kaplan, C.D., Simons, C., and Mattick, J.S. Evolution, biogenesis and function of promoter-associated RNAs. *Cell Cycle* 8, 2332-2338 (2009) DOI: 10.4161/cc.8.15.9154

224. Minajigi, A., Froberg, J., Wei, C., Sunwoo, H., Kesner, B., Colognori, D., Lessing, D., Payer, B., Boukhali, M., Haas, W., Lee, J.T. Chromosomes. A comprehensive Xist interactome reveals cohesin repulsion and an RNA-directed chromosome conformation. *Science* 349 (2015) DOI: 10.1126/science.aab2276

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