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Transcriptional gene silencing mediated by non-coding RNAs

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Abstract

Chromatin remodelling guided by non-coding RNA (ncRNA) contributes mechanistically to the establishment of chromatin structure and to the maintenance of epigenetic memory. Various ncRNAs have been identified as regulators of chromatin structure and gene expression. The widespread occurrence of antisense transcription in eukaryotes emphasizes the prevalence of gene regulation by natural antisense transcripts. Recently, antisense ncRNAs have been implicated in the silencing of tumor suppressor genes through epigenetic remodeling events. Characterization of the antisense RNAs involved in the development or maintenance of oncogenic states may define ncRNAs as early biomarkers for the emergence of cancer, and could have a significant impact on the development of tools for disease diagnosis and treatment. In this review, current knowledge on the mechanisms of ncRNA-mediated transcriptional gene silencing in humans is discussed, and parallels between the establishment of a silent chromatin state mediated by siRNAs and long antisense ncRNAs are highlighted.

Keywords

Antisense transcription; chromatin; epigenetic; non-coding RNA; siRNA; transcriptional gene silencing

Introduction

Transcriptional gene silencing (TGS) mediated by the siRNA pathway has been well described in the single-cell organisms *Schizosaccharomyces pombe* and *Tetrahymena thermophila*, as well as in the more complex organisms *Arabidopsis thaliana*, *Drosophila melanogaster* and *Caenorhabditis elegans* [1,2]. Small RNA-directed TGS has also been observed in human cells following the exogenous delivery of siRNAs targeted to gene promoters [3]. Additional evidence has emerged in recent years that suggests an endogenous RNA-mediated TGS mechanism exists in the nucleus of mammalian cells, acting through long antisense non-coding RNA (ncRNA) transcripts [4–6]. Studies that have investigated transcriptional activity throughout the mammalian genome, predominantly from regions with low or no protein-coding potential [7–17], have revealed an unexpected level of complexity in the mammalian transcriptome. The regulatory potential of non-coding transcription is only beginning to be dissected on a case-by-case basis. In this review, parallels and potential interrelations are discussed between TGS of human genes triggered by exogenously delivered siRNAs, TGS of tumor suppressors triggered by long antisense ncRNAs, and developmentally regulated genome-wide programming by the Polycomb group (PcG) of proteins. These three seemingly distinct phenomena have the underlying common theme of ‘ncRNA-mediated TGS’.

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Mechanism of siRNA-mediated transcriptional gene silencing

Promoter-targeted siRNAs have been demonstrated to inhibit the transcription of various human and viral genes, including *EF1A* [3,18], ubiquitin C (*UBC*) [19], *CCR5* [20], *RASSF1A* [20,21], the progesterone receptor [22–24], the androgen receptor and huntingtin [25], the major vault protein and cyclooxygenase-2 [22], cadherin-1 [26], endothelial nitric oxide synthase [27], oncogene *c-MYC* [28], viral genes regulated by the long terminal repeat region of HIV-1 [18,29,30] and simian immunodeficiency virus [31], and the human, as well as the mouse *INK4/ARF* locus encoding several tumor suppressor proteins [32]. In these studies, exogenously delivered siRNAs with a sequence complementary to the target promoter were capable of triggering epigenetic modifications at the target genomic loci, resulting in the silencing of gene transcription. Importantly, the antisense strand of siRNA alone was able to direct sequence-specific TGS via an increase in the epigenetic marks that are characteristic for silenced chromatin at the target promoter in human cells [18].

Chromatin remodeling in the vicinity and downstream of the initial siRNA target site accompanies RNA-triggered TGS. For example, an enrichment of the silent chromatin marks H3K9me2 (dimethylation at Lys^{9} of histone 3) and H3K27me3 (trimethylation of Lys^{27} at histone 3) was observed at siRNA-targeted promoter loci ([3,18–20,30,32–36]; reviewed in reference [37]). Proteins involved in TGS in humans have not been characterized comprehensively. The RNA-binding protein argonaute 1 (Ago1) has been established to be required for the initiation of siRNA-mediated TGS in human cells [19,20,25,30,32,35,36]. In addition to Ago1, Ago2 [25,32] and TAR-RNA binding protein 2 (TRBP2) [20] were also observed to be involved in siRNA-mediated TGS in humans. For example, Ago2 and Ago1 were involved in small-duplex RNA-mediated TGS of the progesterone receptor promoter in T47D cells [25]. In this particular example, TGS was not accompanied by epigenetic modifications in promoter-associated histones [25]. This study highlighted the possible existence of different RNA-mediated TGS pathways in human cells that may be regulated by different factors, and the observation that TGS may not always be accompanied by chromatin remodeling [25]. However, proteins involved in chromatin remodeling, including the *de novo* DNA methyltransferase Dnmt3A [18,19,30], HDAC1 [19,30,36] and the histone lysine methyltransferases KMT6 (formerly Ezh2) [20,30] and KMT1C (formerly G9a) [19], were demonstrated to play a role in TGS in humans (Figure 1; for the new nomenclature of chromatin-modifying enzymes, see reference [38]). The initial steps in TGS are the recruitment of the Ago1-siRNA nucleoprotein complex to the targeted promoter loci and the interaction of the complex with a promoter-associated nascent RNA transcribed by RNA polymerase II (RNAPII) [34]. Transcripts that are targeted by the TGS pathway in a siRNA- and Ago1-dependent manner in the nucleus are not degraded [37]. Ago1 was also observed to interact with RNAPII [20]. The RNAPII-Ago1 interaction may actively contribute to the recruitment of the Ago1-RNA complex to the gene promoter. An analysis of high-throughput sequencing data of a short-RNA population from mouse embryonic stem (ES) cells and from a genome-wide nuclear run-on assay in human fibroblasts revealed that widespread and divergent transcription occurs at promoters over short distances, and that this phenomenon is common for active genes in higher eukaryotes [39,40]. Depletion of the exosome in human cells identified a new class of upstream transcripts within promoters (PROMPTs) that are transcribed in both directions, and the levels of which peak at distances of 1 kb upstream of known protein-coding genes [41]. These recent genome-wide studies support previous observations that promoter-associated RNA transcripts associate with several genes and play a role in TGS [34]. Although the two silent chromatin marks, H3K9me2 and H3K27me3, together with the Ago1, Dnmt3A and HDAC1 proteins are considered to be hallmarks of siRNA-mediated TGS in human cells [37], the mechanisms of chromatin modification at promoters remain to be determined.

Long-term transcriptional gene silencing

Short hairpin RNA-mediated transcriptional gene silencing

An important aspect of TGS in humans has emerged recently. A short hairpin RNA (shRNA) targeted to the promoter of the *UBC* gene in human cells was demonstrated to mediate long-term transcriptional silencing [19]. Importantly, the suppression of *UBC* was observed for 1 month, although expression of the shRNA targeting the *UBC* promoter was induced for only 7 days. TGS was dependent on the presence of a promoter-targeted antisense shRNA, and additional factors such as Ago1, Dnmt3a and HDAC1, and to some extent the H3K9-specific lysine methyltransferase KMT1C, but not the H3K27-specific lysine methyltransferase KMT6 [19]. Conversely, the maintenance of long-term TGS was dependent on the DNA methyltransferases Dnmt1 and Dnmt3a, but was independent of Ago1, HDAC1 or the shRNA that initially triggered TGS [19]. Methylation of CpG islands in the promoters of human genes has been established to play a significant role in the stable, long-term epigenetic silencing of genes throughout healthy development, and in cancer [42]. In addition, the requirement for Dnmt1, an enzyme responsible for the maintenance of DNA methylation, correlated with the observed increase in DNA methylation at the *UBC* promoter [19]. These data suggest that silencing is established initially at the level of histone methylation, which is subsequently followed by DNA methylation. After these epigenetic changes have occurred, it is possible to maintain TGS in a long-term manner without the need for the initial RNA effector molecule [19]. This example of long-term silencing by a ncRNA is the only published example of long-term TGS by exogenous RNA effectors [19]. Many cases of long-term TGS by endogenous long ncRNAs also exist, and are reviewed in reference [43].

Long non-coding RNA-mediated transcriptional gene silencing

The Polycomb and Thrithorax groups of proteins—Polycomb (PcG) proteins act to repress transcription, whereas the Thrithorax group (TrxG) of proteins act to maintain transcription. These proteins are important genome-wide regulators that are required to establish long-term gene expression during development through modulation of the epigenetic state of chromatin [44,45]. In *D melanogaster*, PcG response elements (PREs) or TrxG response elements (TREs) are several hundred base pairs in length and function as the sites of PcG or TrxG complex recruitment, respectively [44]. The definition of PREs or TREs in vertebrates has not been established [44,46]. Interestingly, many PREs and TREs appear to be transcribed from both DNA strands [46]. Hekimoglu and Ringrose suggest, among four alternative models, one possible model for PcG/TrxG recruitment that is based on an RNA-RNA interaction: a free RNA transcript binds the PcG or TrxG complex; subsequently, the PcG-RNA and/or TrxG-RNA complex associates with a complementary nascent RNA transcript transcribed in the opposite direction, resulting in the recruitment of PcG/TrxG to bidirectionally transcribed genomic loci [46]. An analogous mechanism was proposed for TGS in the case of the p21 and E-cadherin tumor suppressors in human cells [47], whereby antisense RNA transcript spanning a gene promoter targets a nascent promoter-associated RNA transcribed in the sense direction. This targeting results in recruitment of a putative transcriptional silencing complex and subsequent formation of heterochromatin in the promoter region, leading to transcriptional silencing of the target genes [47] (Figure 1).

An example of PcG- and TrxG-regulated genes that are involved in the development of higher eukaryotes is the *HOX* family of genes [44]. In a comprehensive study investigating the transcriptional activity of human *HOX* loci, 231 ncRNA transcripts with low-coding potential were identified to be transcribed from the intergenic regions [48]. The majority of these ncRNA transcripts (74%) were transcribed in an antisense orientation compared with the *HOX* genes [48]. The same study identified a long ncRNA termed HOTAIR (*Hox* antisense intergenic RNA), which is a 2.2-kb spliced and polyadenylated transcript that is transcribed from the

HOXC locus. The HOTAIR transcript negatively regulates the distant *HOXD* locus in trans through the recruitment of the PRC2 Polycomb complex, which correlates with increased levels of the repressive H3K27me3 mark on the *HOXD* locus [48].

Long non-coding RNAs involved in X inactivation—In the case of X chromosome inactivation, a 17-kb ncRNA transcript in humans [49] and a 15-kb transcript in mice [50], the X inactive-specific transcript (Xist) mediates silencing of gene expression from one copy of the X chromosome, the mechanism through which dosage compensation in female mammals operates during early development [51]. A study in mouse ES cells and embryonic fibroblasts led to the characterization of a 1.6-kb RepA long ncRNA that was transcribed from the repeat A element of the *XIST* locus [52]. The RepA ncRNA recruited the PRC2 Polycomb complex to the *XIST* promoter through a direct interaction with the KMT6 subunit of PRC2 [52]. Initial local trimethylation of H3K27 at the 5′ end of the *XIST* gene by PRC2 created a heterochromatic patch that, paradoxically, led to *XIST* transactivation resulting in widespread X inactivation [53]. In mice, *XIST* activity itself was negatively regulated in cis by the 40-kb ncRNA Tsix (X inactive-specific transcript backwards), which is transcribed antisense to *XIST* [54]. Tsix also modulates the chromatin status at the *XIST* promoter [51]. Interestingly, the transcription of *TSIX* through the *XIST* promoter in the antisense direction is a prerequisite for downregulation of *XIST* [55]. Although the Tsix sequence is conserved between mice and humans [54], whether Tsix-mediated downregulation of *XIST* occurs in human cells remains to be determined. These recent insights into the mechanism of X inactivation (reviewed in references [51,56,57]) revealed a complex role for long ncRNAs in the regulation of gene expression through modulation of the epigenetic status.

Genomic imprinting of autosomal genes—Additional examples of long ncRNAs involved in genomic imprinting of autosomal genes – a mono-allelic mechanism of gene silencing based on the parent-of-origin- include Air [58] and Kcnq1ot1 [59] (reviewed in reference [57]); this mechanism has been studied extensively in mice. Both the 108-kb *Air* and the 91-kb Kcnq1ot1 ncRNAs are transcribed by RNAPII [60,61]. These ncRNAs function to silence large domains of the genome epigenetically through their interaction with chromatin. The Air ncRNA silences in cis the three paternally inherited genes *IGF2R*, *SLC22A2* and *SLC22A3*, which are all within 300 kb, and is transcribed in an antisense direction to *IGF2R* and *SLC22A3* [58]. Air localizes to the silenced *SLC22A3* promoter and recruits the KMT1C lysine methyltransferase, which leads to targeted H3K9 methylation and allele-specific gene silencing by chromatin remodeling [60]. The Kcnq1ot1 mRNA is transcribed from intron 10 of the *KCNQ1* gene in an antisense direction and silences several paternally inherited genes in cis that are transcribed in both directions from within a 780-kb region [59,61–63]. Kcnq1ot1 RNA itself is required for epigenetic silencing of neighboring genes upstream and downstream of the *KCNQ1* locus [64]. In addition, epigenetic silencing has been demonstrated to correlate with the interaction of Kcnq1ot1 with both the PRC2 Polycomb complex and the KMT1C lysine methyltransferase, as well as with the enrichment of the repressive histone modifications H3K27me3 and H3K9me3 at the loci of silenced genes [64]. The PRC2 complex has been identified to be responsible for trimethylation of H3K27 in *D melanogaster* [65], while KMT1C is responsible for mono- and dimethylation of H3K9 at Kcnq1ot1-targetted promoters in mammalian cells [66]. The mechanism of epigenetic gene silencing by long ncRNAs in X inactivation and in genomic imprinting was recently reviewed comprehensively by Nagano and Fraser [57],

Interestingly, while Air localizes to the nucleus, spliced versions of this ncRNA have been demonstrated to localize in the cytoplasm [67]. In comparison, Kcnq1ot1 is polyadenylated, but not spliced, and localizes exclusively to the nucleus [64]. Introducing an exon/intron boundary into the *KCNQ1* gene had no observable effect on the nuclear localization of Kcnq1ot1 RNA [64]. Intriguingly, the HOTAIR antisense RNA, which regulates the *HOXD*

cluster of genes, is both spliced and polyadenylated [48]. Based on these observations, a striking aspect of ncRNA biogenesis, that has not yet been assessed comprehensively, is the role of post-transcriptional processing and the potential influence of this process on the biological activity and nuclear localization of regulatory ncRNAs.

Transcriptional gene silencing of tumor suppressors in human cells is mediated by antisense transcription

Many tumor suppressor genes are silenced epigenetically in a variety of cancers [42]. In addition, various genes associated with CpG islands also undergo *de novo* DNA methylation in cancer [42]. Yu *et al* recognized that several annotated antisense transcripts are associated with many well-characterized tumor suppressor genes, and investigated the possibility that antisense RNAs may trigger epigenetic silencing of mammalian genes [68]. In this study, the p15 antisense RNA transcript was demonstrated to silence p15 expression both in cis and in trans, although the cis mechanism predominated. Interestingly, eliminating overexpression of p15 antisense RNA did not reactivate p15 mRNA expression, indicating that long-term transcriptional silencing was established upon exposure to high levels of the p15 antisense RNA. In addition, relatively stable silencing of p15 expression correlated with an increase in H3K9me2 and with a decrease in the transcriptionally active chromatin mark H3K4me2 at the p15 promoter. However, an increase in p15 promoter DNA methylation was not observed, suggesting that the formation of heterochromatin rather than DNA methylation of the p15 promoter was a prerequisite for p15 antisense RNA-mediated TGS. Moreover, TGS of p15 induced by the antisense transcript was Dicer-independent, indicating that the mechanism of p15 silencing did not act through the siRNA/miRNA post-transcriptional gene-silencing pathway, in which the Dicer complex is a central component [68].

Although most data on the role of siRNAs in TGS demonstrate the ability of these molecules to silence target gene promoters directly via the initiation of epigenetic changes, treatment of human cells with siRNAs targeted toward promoter regions also resulted in the transcriptional activation of E-cadherin and the tumor suppressor p21 genes [69,70]. A subsequent study conducted to determine the mechanism of siRNA-mediated activation of p21 gene transcription indicated that the activating siRNAs targeted and post-transcriptionally silenced an antisense ncRNA transcript associated with the p21 genomic locus [47]. This study therefore revealed that antisense ncRNAs can negatively regulate their sense counterpart and function as effectors of TGS in human cells in an Ago1-dependent manner [47]. These data indicated that RNAs transcribed in an antisense direction can lead to an enrichment of H3K9me2 and H3K27me3 at the sense protein-coding promoter, resulting in TGS. In the case of siRNA-mediated TGS, the antisense strand of siRNA alone is the effector molecule that triggers TGS in human cells [18]. Thus, while the studies with siRNAs highlighted potential mechanisms of action, it is the antisense RNAs that have helped elucidate the underlying mechanism of TGS in human cells [18]. The direct consequence of an imbalance in endogenous bidirectional transcription could potentially lead to the deregulation of genes, a situation often observed in various diseases, including cancer. These data, when considered in the context of previously described data on long ncRNAs that function in imprinting, such as Xist, Air and Kcnq1ot1, suggest that the association of antisense ncRNA transcripts that have a regulatory function with the transcription of protein-coding genes in human cells could be a more common phenomenon than previously anticipated.

Chromatin-modifying activities potentially involved in ncRNA-mediated transcriptional gene silencing

KMT6, and the recently identified mammalian homolog Ezh1 [71,72], are the only known methyltransferases that methylate H3K27 (a transcriptionally repressive chromatin mark that is associated with TGS). KMT6 and/or Ezh1 are associated with two other subunits, SUZ12 (JJAZ1) and EED, in the PRC2 and/or PRC3 Polycomb complexes [73,74], which have been implicated in mediating long-term TGS in mammals (discussed in detail in the *Polycomb and Thrithorax groups of proteins* section). Moreover, an interaction between the HDAC1 and HDAC2 histone deacetylases and the EED subunit of the PRC2 Polycomb complex was demonstrated in which histone deacetylase activity was required for PRC2-mediated transcriptional repression, thereby providing a mechanistic link between Polycomb-mediated gene repression and histone deacetylation [75]. In a subsequent study, an important physical and functional interaction between KMT6 and the DNA methyltransferases Dnmt1, Dnmt3A and Dnmt3B in the context of the PRC2 Polycomb complex was demonstrated, whereby KMT6 was required for CpG methylation of Polycomb-targeted promoters [76]. The requirement for the H3K27me3-specific KMT6 lysine methyltransferase in some cases, together with the requirement of HDAC1 and Dnmt3a for establishing TGS [37], strongly suggest a role for the PRC2 and/or possibly the PRC3 Polycomb complex in RNA-mediated TGS. Although H3K27me3 is undoubtedly linked to TGS, the precise mechanistic role of H3K27-specific lysine methyltransferase activity in association with the PCR2 and/or PCR3 Polycomb complexes is unclear, and requires further investigation.

Methylation of Lys^9 of histone H3 (H3K9) is an epigenetic hallmark of heterochromatin formation and transcriptional silencing in eukaryotes [77]. Five lysine methyltransferases have been identified for which H3K9 is a substrate: KMT1C (G9a), KMT1D (Glp), KMT8 (Riz1), KMT1E (Eset) and KMT1A/B (Suv39h1/2) ([38]; reviewed in reference [78]). Of these five methyltransferases, KMT1C appears to be the predominant H3K9 methyltransferase in mammalian cells [79,80]. A functional interaction between KMT1C-mediated H3K9 methylation and DNA methylation was demonstrated in several studies [81–84]. In particular, a direct interaction between the histone methyltransferase KMT1C and the DNA methyltransferase Dnmt1 was reported [82]. In ES cells, the KMT1C/KMT1D complex was demonstrated to regulate both H3K9 incorporation and DNA methylation, two epigenetic marks that cooperatively silence gene expression [85]. Furthermore, genetic evidence has indicated that the DNA methyltransferases Dnmt3A and Dnmt3B are responsible for *de novo* DNA methylation at the KMT1C/KMT1D target loci [85]. These data, together with the observations that KMT1C is required for the long-term silencing of the *UBC* promoter [19], suggest that KMT1C is the primary candidate for H3K9-specific methyltransferase activity in human cells, and should be investigated further.

The DNA methyltransferases Dnmt3A and Dnmt1 had been demonstrated to associate *in vivo* with the histone deacetylase HDAC1 [86–89] and with the H3K9-specific methyltransferase KMT1A [90]. Thus, given the complex network of interactions between chromatin-modifying proteins, a putative transcriptional silencing complex may mediate TGS in the nucleus of human cells. As outlined in Figure 1, the transcriptional silencing complex may contain either the KMT1C or KMT1A H3K9-specific methyltransferases and other chromatin-modifying proteins, such as the KMT6-containing Polycomb complex PRC2, which methylates H3K27, HDAC1 and the DNA methyltransferases Dnmt3A and Dnmt1. A comprehensive characterization of the enzymatic activities responsible for the chromatin remodeling that accompanies TGS, and further biochemical analysis of protein complexes involved in TGS in the cell nucleus will be invaluable for gaining insight into the mechanism of RNA-mediated TGS.

Parallels between transcriptional gene silencing in development, imprinting and cancer

Currently, limited data are available on antisense RNA-mediated TGS in the context of tumor suppressor proteins [47,68]. Nevertheless, studies on TGS of tumor the suppressors p15 and p21 [47,68] suggest that the mechanism of TGS closely resembles that of developmentally regulated and imprinted genes, whereby long ncRNAs function in cis in combination with Polycomb proteins to establish a silent chromatin state and to silence gene expression [47, 68]. The analogy between Polycomb-mediated gene silencing in development and antisense-mediated epigenetic silencing of tumor suppressor genes in cancer suggests that there is a common underlying theme among these apparently distinct biological processes. Therefore, targeting the epigenetic machinery, which involves Polycomb protein complexes and perhaps DNA methyltransferase complexes, to the epigenetically silenced genes during both development and cancer could be mediated by a related mechanism involving long ncRNA transcripts in higher eukaryotes [6].

Evidence suggesting that the well-characterized tumor suppressor genes encoded in the *INK4a/ARF* locus, which control cell-cycle progression and senescence, are regulated by Polycomb proteins was obtained in a study in mice by Jacobs *et al* [91]. Since this study, the regulation of the *INK4a/ARF* tumor suppressor genes has been investigated extensively and the epigenetic mode of their transcriptional regulation is well established (reviewed in references [92,93]). A long antisense ncRNA termed ANRIL was detected within the *INK4a/ARF* chromosomal region in human cells; the first exon of ANVIL is located in the promoter of the *p14/ARF* gene and overlaps two exons of the *p15/CDKN2B* gene [94]. The role of ANRIL or potentially other, as yet unidentified, ncRNAs in the regulation of the *INK4a/ARF* locus has currently not been established.

Interestingly, in some human cells, including the differentiated fibrosarcoma cell line HT1080 [95] and immature hematopoietic precursor cells [96], the induction of X inactivation was Xist-responsive outside of the usual discrete developmental window in which expression if the *XIST* gene normally occurs [56]. In addition, in a mouse thymic lymphoma model, the induction of Xist resulted in the initiation of X inactivation and the inhibition of tumor growth [97]. This study also led to the identification of SATB1, a nuclear matrix protein and genome organizer [98], and its close homolog SATB2[97] as the first identified cofactors required for Xist-mediated initiation of X chromosome inactivation [97]. These findings suggest that perturbing mechanisms of TGS involving Xist ncRNA play a significant role in tumorigenesis. Moreover, elevated expression levels of SATB1 correlated with aggressive breast tumor phenotypes, whereby SATB1 upregulated metastasis-associated genes and downregulated tumor suppressor genes directly [99]. The role of SATB1 in both X chromosome inactivation and tumorigenesis is notable, and future studies are expected to reveal whether the SATB1/SATB2 proteins have a more general role in RNA-mediated TGS.

Whether long ncRNAs, ubiquitous antisense transcription and Polycomb complexes mediate transcriptional gene silencing in development, imprinting and cancer in humans remains to be established. A better understanding of the TGS mechanism mediated by ncRNAs through epigenetic changes in chromatin will undoubtedly help to resolve these issues.

Conclusion

Transcription in the mammalian genome is pervasive, both in the sense and antisense directions. Emerging evidence highlights a role and function for ncRNAs in the regulation of gene expression. While there are clearly differences between the experimental systems discussed herein, namely siRNA-mediated TGS, Polycomb-mediated silencing of imprinted

genes and antisense RNA-mediated repression of tumor suppressors, a common underlying regulatory theme, whereby ncRNAs silence gene transcription through epigenetic modifications of chromatin within promoter regions, is evident. Only a few proteins involved in ncRNA-mediated TGS pathways have been identified and attempts to characterize the underlying ncRNA-mediated TGS mechanism in human cells are at an early stage. A detailed mechanistic understanding of RNA-mediated TGS of tumor suppressors will contribute significantly to the understanding of the molecular mechanisms that underlie the early stages of tumor development. Understanding the TGS mechanism on a molecular level will lead directly to molecular strategies aimed at preventing tumorigenesis. Restoring physiological levels of gene expression for tumor suppressors by suppressing the transcription of antisense ncRNAs in a highly specific manner, using RNAi technology, has significant therapeutic potential, providing a promising strategy for the treatment of cancer and other diseases by the targeted control of gene transcription. The identification of a comprehensive set of genes that appear to be silenced by antisense ncRNAs in various tumor tissues will prove to be a highly valuable prognostic biomarker for the emergence of cancer.

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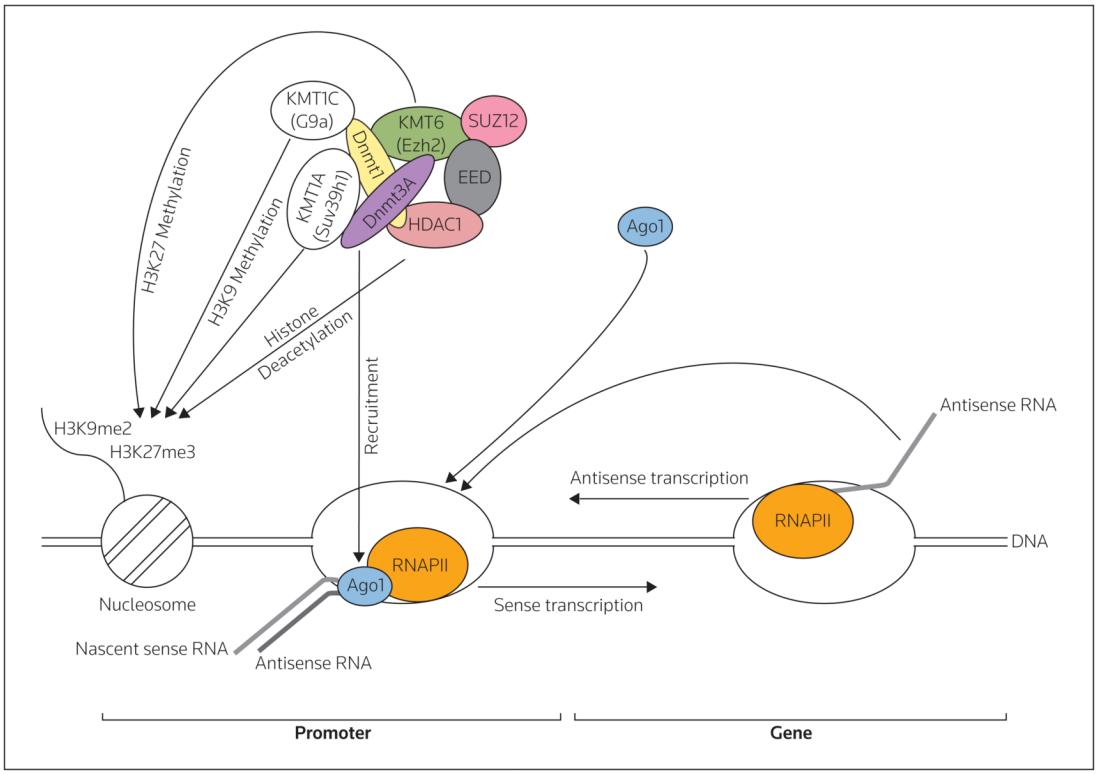

Figure 1. A model for the antisense non-coding RNA-mediated initiation of transcriptional gene silencing in human cells

Antisense RNA associates with the argonaute 1 (Ago1) protein. The RNA-Ago1 complex then targets the nascent promoter-associated RNA, which is transcribed by RNA polymerase II (RNAPII) in the sense direction. Subsequently, the putative silencing complex, which may consist of the PRC2 Polycomb complex (composed of KMT6 [Ezh2], SUZ12 and EED), HDAC1, the DNA methyltransferases Dnmt3A and Dnmt1 and the histone methyltransferases KMT1C (G9a) and/or KMT1A (Suv39h1), is recruited to the promoter. Recruitment of the silencing complex may be mediated through the interaction of Ago1 with Dnmt3a, or directly through promoter-associated RNA. HDAC1-mediated histone deacetylation may precede histone H3 methylation at Lys^{9} and Lys^{27} (H3K9me2 and H3K27me3, respectively) of the nucleosomes proximal to the promoter target site. Histone methylation may be mediated by the candidate lysine methyltransferases KMT6 (for H3K27) and KMT1C (for H3K9) and/or KMT1A (alternative H3K9 methyltransferases are shown in white), leading to the formation of heterochromatin at the target promoter. The associations of protein subunits indicate known interactions.