NON-CODING RNA

Non-coding RNAs in human disease

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Abstract | The relevance of the non-coding genome to human disease has mainly been studied in the context of the widespread disruption of microRNA (miRNA) expression and function that is seen in human cancer. However, we are only beginning to understand the nature and extent of the involvement of non-coding RNAs (ncRNAs) in disease. Other ncRNAs, such as PIWI-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), transcribed ultraconserved regions (T-UCRs) and large intergenic non-coding RNAs (lincRNAs) are emerging as key elements of cellular homeostasis. Along with microRNAs, dysregulation of these ncRNAs is being found to have relevance not only to tumorigenesis, but also to neurological, cardiovascular, developmental and other diseases. There is great interest in therapeutic strategies to counteract these perturbations of ncRNAs.

Imprinting
The epigenetic marking of a gene on the basis of parental origin, which in somatic tissues results in monoallelic expression.

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The most well-studied sequences in the human genome are those of protein-coding genes. However, the coding exons of these genes account for only 1.5% of the genome, a proportion that increases to 2% if untranslated regions (UTRs) are considered[1]. In recent years, it has become increasingly apparent that the non-protein-coding portion of the genome is of crucial functional importance: for normal development and physiology and for disease[2]. The functional relevance of the non-protein-coding genome is particularly evident for a class of small non-coding RNAs (ncRNAs) called microRNAs (miRNAs)[3,4]. In human diseases, particularly cancer, it has been shown that epigenetic and genetic defects in miRNAs and their processing machinery are a common hallmark of disease[5–8]. However, miRNAs are just the tip of the iceberg, and other ncRNAs, such as transcribed ultraconserved regions (T-UCRs), small nucleolar RNAs (snoRNAs), PIWI-interacting RNAs (piRNAs), large intergenic non-coding RNAs (lincRNAs) and, overall, the heterogeneous group of long non-coding RNAs (lncRNAs), might also contribute to the development of many different human disorders[2].

Here, I focus on the genetic and epigenetic events that disrupt ncRNA loci and their related proteins in the context of cancer and other human diseases, such as neurological, cardiovascular, autoimmune, imprinting and monogenic disorders. I also discuss the emerging opportunities for targeting these disruptions of ncRNAs using novel therapeutic approaches. The role of miRNAs in cancer has recently been reviewed in detail elsewhere[5–8], and so it is only briefly recapped here. By contrast, this Review considers in more depth the emerging evidence for roles of other ncRNAs in cancer and for both miRNAs and other ncRNAs in other types of disease.

Types of ncRNA and their functions

The discovery that many genomic sequences in complex organisms are transcribed in a developmental- and tissue-regulated fashion[9,10] has fuelled a race to characterize all of the different types of ncRNAs that are transcribed in human cells. Although most of the work has focused on short RNAs, such as miRNAs, lncRNAs are also gaining prominence. In the case of lncRNAs, they are classified as those ncRNAs that are longer than 200 nucleotides on the basis of RNA purification protocols that exclude small RNAs[10]. Although there is not necessarily a clear delineation between ncRNA classes, for the purpose of simplicity, in this Review, I discuss ncRNAs by division into the following categories: miRNAs, piRNAs, snoRNAs, lncRNAs (for example, homeobox (HOX) transcript antisense RNA (*HOTAIR*), lincRNAs and T-UCRs) and other types of ncRNAs. TABLE 1 summarizes the different types of ncRNAs that are discussed, and FIG. 1 illustrates the biogenesis machineries of the most well-characterized ncRNAs.

miRNAs. The most widely studied class of ncRNAs are miRNAs, which are small ncRNAs of ~22 nucleotides (nt) that, in animals, mediate post-transcriptional gene silencing by controlling the translation of mRNA into proteins[3,4]. miRNAs are estimated to regulate the translation of more than 60% of protein-coding genes. They are involved in regulating many processes, including proliferation, differentiation, apoptosis and

Table 1 | **Types of ncRNAs***

Name	Size	Location	Number in humans	Functions	Illustrative examples	Refs
Short ncRNAs						
miRNAs	19–24 bp	Encoded at widespread locations	>1,424	Targeting of mRNAs and many others	miR-15/16, miR-124a, miR-34b/c, miR-200	3–8
piRNAs	26–31bp	Clusters, intragenic	23,439	Transposon repression, DNA methylation	piRNAs targeting *RASGRF1* and LINE1 and IAP elements	13–19
tiRNAs	17–18bp	Downstream of TSSs	>5,000	Regulation of transcription?	Associated with the *CAP1* gene	37
Mid-size ncRNAs						
snoRNAs	60–300 bp	Intronic	>300	rRNA modifications	U50, SNORD	20–22
PASRs	22–200 bp	5′ regions of protein-coding genes	>10,000	Unknown	Half of protein-coding genes	10
TSSa-RNAs	20–90 bp	–250 and +50 bp of TSSs	>10,000	Maintenance of transcription?	Associated with *RNF12* and *CCDC52* genes	35
PROMPTs	<200 bp	–205 bp and –5 kb of TSSs	Unknown	Activation of transcription?	Associated with *EXT1* and *RBM39* genes	36
Long ncRNAs						
lincRNAs	>200 bp	Widespread loci	>1,000	Examples include scaffold DNA–chromatin complexes	*HOTAIR*, *HOTTIP*, *lincRNA-p21*	2,28–30
T-UCRs	>200 bp	Widespread loci	>350	Regulation of miRNA and mRNA levels?	uc.283+, uc.338, uc160+	31–34
Other lncRNAs	>200 bp	Widespread loci	>3,000	Examples include X-chromosome inactivation, telomere regulation, imprinting	*XIST*, *TSIX*, TERRAs, *p15AS*, *H19*, *HYMAI*	2,23–25

*There is not necessarily a clear delineation between classes of non-coding RNA (ncRNA); for example, X-inactivation specific transcript (*XIST*) and its antisense transcript *TSIX* could be considered as large intergenic non-coding RNAs (lincRNAs). In the 'Location' column, '–' represents the number of base pairs upstream of the transcription start site (TSS) and '+' represents the number of base pairs downstream of the TSS. *CAP1*, CAP, adenylate cyclase-associated protein 1; *CCDC52*, coiled-coil domain containing 52 (also known as *SPICE1*); *EXT1*, exostosin 1; *HOTAIR*, homeobox (HOX) transcript antisense RNA; *HOTTIP*, HOXA distal transcript antisense RNA; *HYMAI*, hydatidiform mole associated and imprinted; IAP, intracisternal A-particle; lncRNA, long non-coding RNA; miRNAs, microRNAs; piRNAs, PIWI-interacting RNAs; PASRs, promoter-associated small RNAs; PROMPTs, promoter upstream transcripts; *RASGRF1*, RAS-protein-specific guanine nucleotide-releasing factor 1; *RBM39*, RNA-binding motif protein 39; *RNF12*, ring finger protein 12 (also known as *RLIM*); snoRNAs, small nucleolar RNAs; TERRAs, telomeric repeat containing RNAs; tiRNAs, transcription initiation RNAs; TSSa-RNAs, TSS-associated RNAs; T-UCRs, transcribed ultraconserved regions.

development. Whereas some miRNAs regulate specific individual targets, others can function as master regulators of a process, so key miRNAs regulate the expression levels of hundreds of genes simultaneously, and many types of miRNAs regulate their targets cooperatively[3,4]. Biogenesis of miRNAs takes place through a multi-step process that involves the RNase III enzymes Drosha and Dicer and ultimately results in the production of mature miRNAs of ~22 nt[11]. These molecules are loaded by the Dicer–TARBP2 (TAR RNA-binding protein 2; also known as TRBP) complex into a member of the Argonaute protein subfamily to form the RNA-induced silencing complex (RISC), of which Argonaute proteins are the catalytic endonuclease components. RISC directs the regulation of mRNA by recognizing a complementary sequence in the targeted mRNA, which is generally located in the 3′UTR. Both the loading of miRNAs into RISC[12] and the function of miRNA machinery are tightly regulated[11]. Translation of mRNA into proteins is repressed by miRNAs by two main means: mRNA degradation and the inhibition of translation initiation[3,4].

Argonaute protein
A type of protein that binds to small RNAs and is the defining component of the RNA-induced silencing complex (RISC). Proteins of this type have an ssRNA-binding domain (PAZ) and a ribonuclease domain (PIWI). The small RNAs guide Argonaute proteins to target mRNAs in order to mediate post-transcriptional degradation and/or translational silencing.

piRNAs. piRNAs are ncRNAs of 24–30 nt in length, are Dicer-independent and bind the PIWI subfamily of Argonaute family proteins that are involved in maintaining genome stability in germline cells[13,14]. They are transcribed from regions in the genome that contain transcribed transposable elements and other repetitive elements. The complex that is formed by piRNAs and PIWI proteins suppresses transposable element expression and mobilization by two different mechanisms that have been described in *Drosophila melanogaster*: cleavage of transposable element transcripts by PIWI proteins — a process that is mediated through base-pairing recognition by the piRNA[15] — and heterochromatin-mediated gene silencing[16]. Three major PIWI-class proteins (namely, PIWIL1, PIWIL2 and PIWIL4) are involved in a so-called 'ping-pong' amplification cycle, creating antisense piRNAs that are capable of repressing the transcript of origin[17]. Interestingly, PIWI proteins are not only involved in direct regulation by degradation but have also been linked to DNA methylation[13,18]. Consistently with this, a single piRNA was recently reported to mediate locus-specific methylation of an imprinted region[19].

snoRNAs. snoRNAs are intermediate-sized ncRNAs (60–300 bp). They are components of small nucleolar ribonucleoproteins (snoRNPs), which are complexes that are responsible for sequence-specific 2′-*O*-methylation[20] and pseudouridylation[21] of ribosomal RNA (rRNA). Post-transcriptional modifications of rRNAs take place

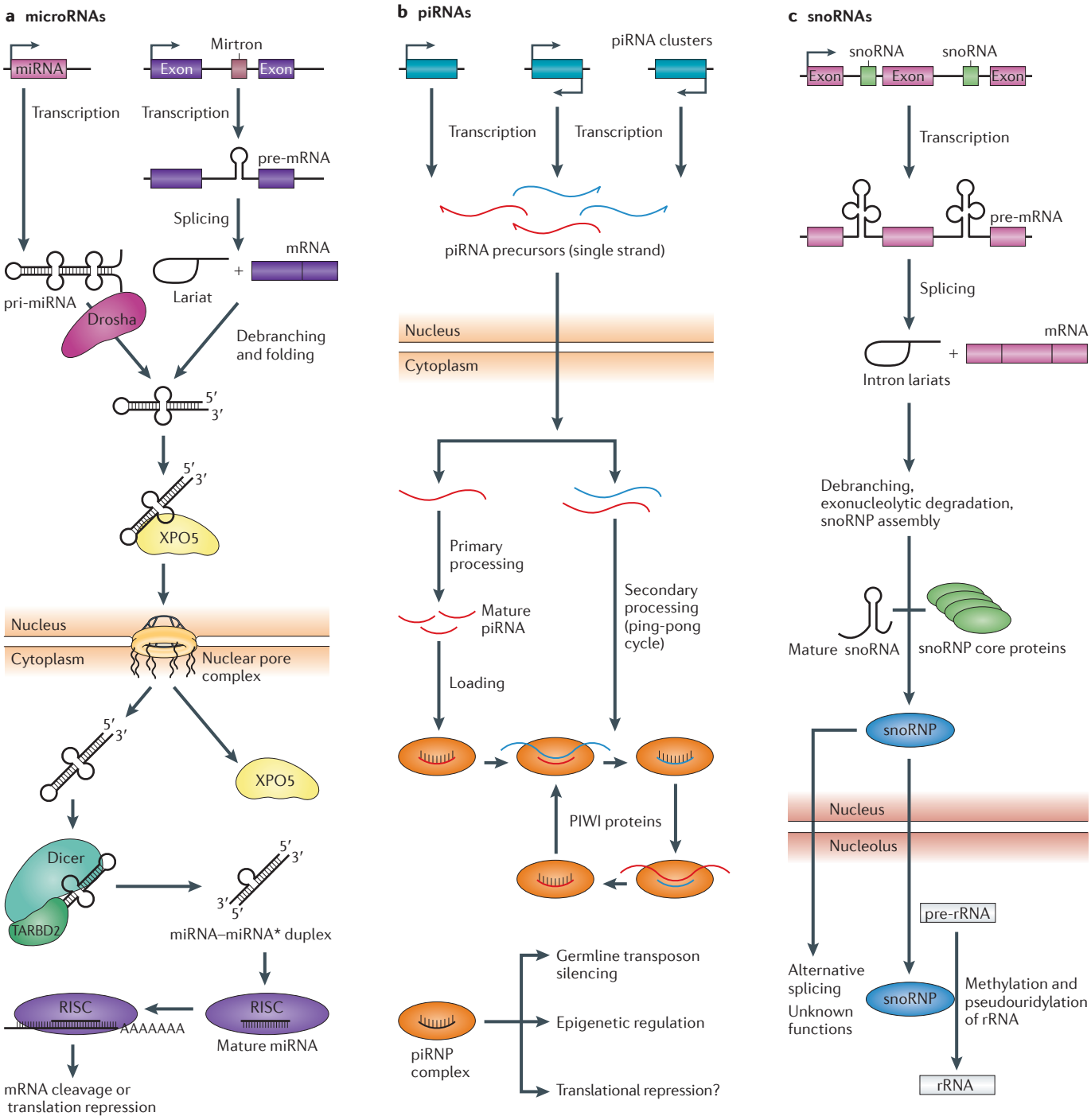

Figure 1 | **The biogenesis and effector machineries of miRNAs, piRNAs and snoRNAs. a** | MicroRNAs (miRNAs) are transcribed as individual units (primary miRNA (pri-miRNA)) or together with host genes (mirtrons). Following processing by the Drosha complex or the lariat-debranching enzyme, respectively, precursor miRNAs (pre-miRNAs) are exported from the nucleus by exportin 5 (XPO5). Further processing by Dicer and TAR RNA-binding protein 2 (TARBP2) generates mature miRNAs, which are loaded into the RNA-induced silencing complex (RISC). miRNAs function through degradation of protein-coding transcripts or translational repression. **b** | PIWI-interacting RNAs (piRNAs) are mainly expressed as ssRNAs from mono- or bidirectional clusters. Subsequently, additional piRNAs are produced through a PIWI-protein-catalysed amplification loop (called the 'ping-pong cycle') via sense and antisense intermediates. The PIWI ribonucleoprotein (piRNP) complex functions in transposon repression through target degradation and epigenetic silencing. **c** | Small nucleolar RNAs (snoRNAs) are predominantly located in introns. Following splicing, debranching and trimming, mature snoRNAs are either exported, in which case they function in ribosomal RNA (rRNA) processing, or remain in the nucleus, where they are involved in alternative splicing and additional yet unknown functions. pre-mRNA, precursor mRNA; snoRNP, small nucleolar ribonucleoprotein.

in the nucleolus — the nuclear compartment within which ribosomes are formed — and facilitate rRNA folding and stability[22]. The sequences of snoRNAs are responsible for targeting the assembled snoRNPs to a specific target.

lncRNAs. lncRNAs are a heterogeneous group of non-coding transcripts more than 200 nt long that are involved in many biological processes. This class of ncRNA makes up the largest portion of the mammalian non-coding transcriptome[2]. Various mechanisms of transcriptional regulation of gene expression by lncRNAs have been proposed. Among these, lncRNAs are known to mediate epigenetic modifications of DNA by recruiting chromatin remodelling complexes to specific loci[23,24]. At the human HOX loci, there is sequential temporal and spatial expression of hundreds of lncRNAs, which regulate chromatin accessibility in a process that involves histone modification enzymes and RNA polymerase[25]. lncRNAs are essential in many physiological processes, such as X-chromosome inactivation in mammals, in which the X-inactivation specific transcript (*XIST*) lncRNA (17 kb) recruits the polycomb complex to silence the X chromosome from which it is transcribed[26]. *TSIX*, another lncRNA, is transcribed from the opposite strand to *XIST* and regulates *XIST* levels during X-chromosome inactivation[23]. As another example, many lncRNAs are expressed by imprinted loci, at which they have a central functional role[2].

Another class of lncRNAs are lincRNAs, which are transcribed from intergenic regions. These transcripts are identified by searching for chromatin signatures that are associated with active transcription in the regions across which transcriptional elongation takes place[27]. Key roles for lincRNAs in certain biological processes are starting to emerge: for example, in the p53-mediated transcriptional response to DNA damage[28]. One p53-regulated lincRNA (termed lincRNA-p21 because it is located near the p21 gene) has a p53-binding motif in its promoter. When p53 induction takes place following DNA damage, this lincRNA represses the expression of downstream genes in the p53 pathway. lincRNAs are being found to regulate both the expression of neighbouring genes[28] and distant genomic sequences[29].

A final class of lncRNAs is those that are transcribed from ultraconserved regions (UCRs). UCRs are DNA segments that are longer than 200 bp and that are completely conserved in human, rat and mouse genomes and most of which are also conserved in chickens and dogs[30]. They are thought to date from a very early period in vertebrate evolution, as most of them have no orthologues in sea squirts, flies or worms[31]. There are 481 described UCRs, some of which overlap with coding exons, although it is believed that more than half of them do not encode any protein[32]. Surprisingly, 68% of UCRs (that is, 325 of them) are transcribed, constituting a new category of ncRNAs, the T-UCRs[33]. Although UCRs range from 200 bp to 779 bp in length, the transcription units of T-UCRs (the non-spliced, full-length cDNAs) are usually up to 2 kb for known T-UCRs[33,34]. T-UCRs are expressed in normal tissues both ubiquitously or in a specific pattern, depending on the tissue[33,34]. The functions of T-UCRs are unknown, but it has been shown that some of them bind to miRNAs[33].

Other types of ncRNAs. Many classes of ncRNA have been described that are associated with the transcriptional start sites (TSSs) of genes: for example, promoter-associated small RNAs (PASRs)[10], TSS-associated RNAs (TSSa-RNAs)[35], promoter upstream transcripts (PROMPTs)[36] and transcription initiation RNAs (tiRNAs)[37]. For all of these classes, their biological functions are poorly defined, although they are probably involved in transcription regulation. Finally, a specialized type of lncRNA, known as telomeric repeat-containing RNAs (TERRAs), is transcribed from telomeres. TERRAs help to maintain the integrity of telomeric heterochromatin by regulating telomerase activity[38].

Disruption of ncRNAs in cancer

Disruption of miRNAs in cancer. The roles of ncRNAs in tumorigenesis have most thoroughly been studied with respect to miRNAs[5–8]. In human cancer, miRNA expression profiles differ between normal tissues and the tumours that are derived from them and differ between tumour types. miRNAs can act as oncogenes or as tumour suppressors and can have key functions in tumorigenesis[5–8]. The example of the miR-200 family in the regulation of epithelial-to-mesenchymal transition is shown in FIG. 2. Dysregulation of miRNAs in cancer can occur through epigenetic changes (for example, promoter CpG island hypermethylation in the case of the miR-200 family[39]) and genetic alterations, which can affect the production of the primary miRNA transcript, their processing to mature miRNAs and/or interactions with mRNA targets (TABLE 2). From a genetic standpoint, one of the first associations to be observed between miRNAs and cancer development was miR-15 and miR-16 dysregulation in most B cell chronic lymphocytic leukaemias as a result of chromosome 13q14 deletion[40]. Interestingly, miRNAs are frequently located in fragile regions of the chromosomes that are involved in ovarian and breast carcinomas and melanomas[41–42].

The recent demonstration of tumour-specific genetic defects in the miRNA-processing machinery, such as in the genes that encode TARBP2 (REF. 43), DICER1 (REF. 44) and exportin 5 (XPO5)[45], has mostly strongly highlighted the relevance of these pathways in cellular transformation, in which such defects contribute to explaining the miRNA dysregulation in cancer. In this regard, although specific miRNAs have been described as acting as oncogenes and tumour suppressors, the miRNA expression profile of human tumours is characterized by a general defect in miRNA production that results in global miRNA downregulation[9].

Disruption of piRNAs in cancer. piRNAs and piRNA-like transcripts have been found to be involved in tumorigenesis in testicular tissue and a range of other tumour types[46–49], although their specific functions in tumorigenesis are unknown. PIWI proteins

Polycomb complex
Originally described in *Drosophila melanogaster*, polycomb complexes maintain the stable and heritable repression of several genes, including the homeotic genes.

Orthologues
Pairs of single genes in two different species that are descended from the same ancestral gene.

Fragile regions
Chromosomal loci that appear as regions of decondensed or partially broken mitotic chromosomes under specific karyotyping conditions.

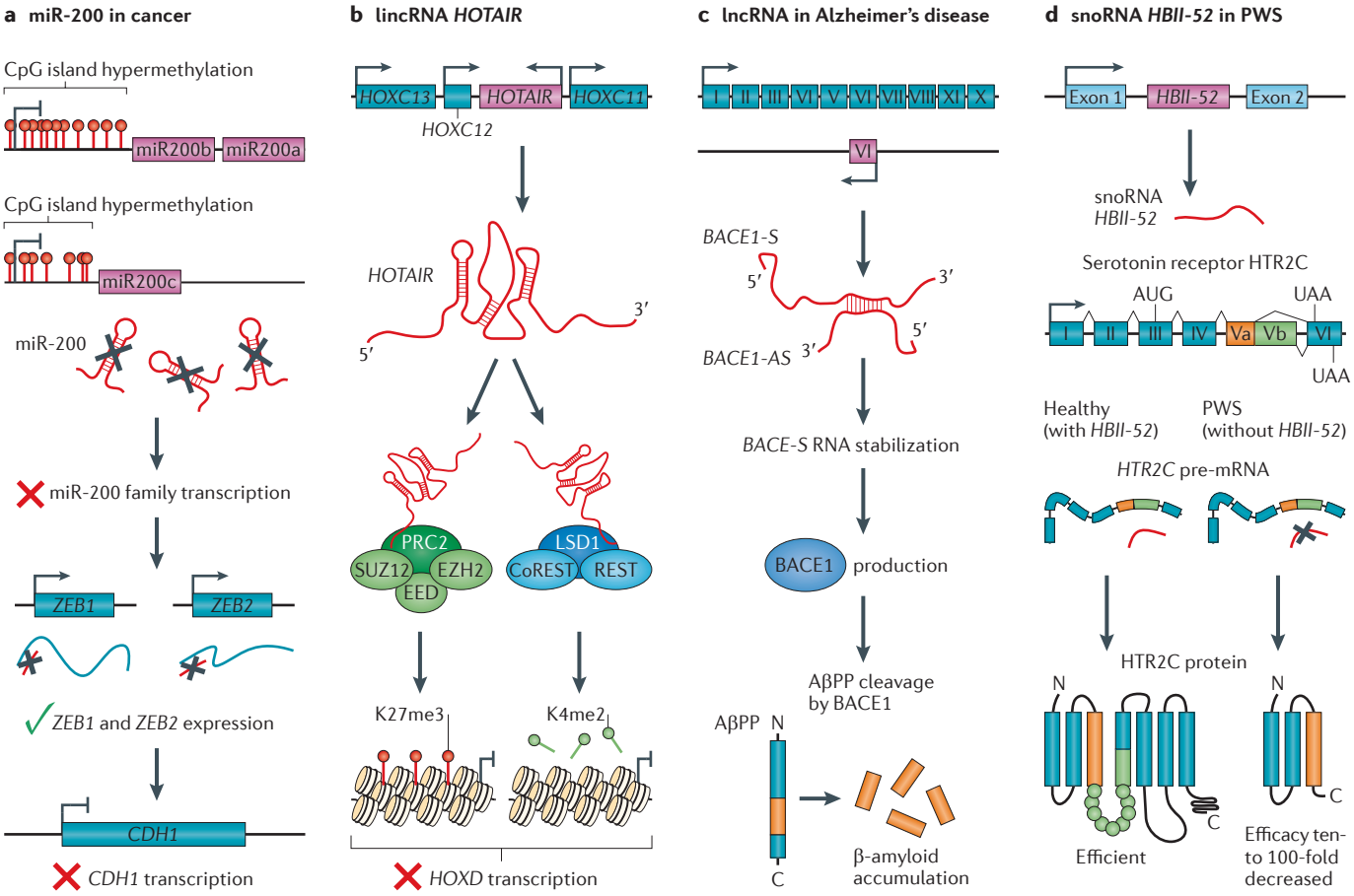

Figure 2 | **Examples of roles of ncRNAs in disease pathophysiology. a** | miR-200 is an example of a microRNA (miRNA) whose role in cancer is well characterized. Alterations in the epigenetic regulation of the miR-200 family are involved in epithelial-to-mesenchymal transition (EMT) in cancer. Specifically, CpG island hypermethylation-associated silencing of these miRNAs in human tumours causes an upregulation of the zinc finger E-box-binding homeobox (HOX) 1 (ZEB1) and ZEB2 transcriptional repressors, which, in turn, leads to a downregulation of E-cadherin (CDH1) — these are changes that promote EMT. **b** | The lincRNA HOX transcript antisense RNA (*HOTAIR*) is involved in polycomb retargeting across the genome. *HOTAIR* expression is increased in transformed cells and induces a genome-wide promoter re-occupancy by polycomb- and H3K27-trimethylation-associated silencing of target genes, such as the HOX protein *HOXD*. The effect of these changes is to increase cancer invasiveness. **c** | lncRNA targeting of β-secretase 1 (BACE1) has a role in the pathophysiology of Alzheimer's disease. An antisense lncRNA, *BACE1-AS*, regulates the expression of the sense *BACE1* gene (labelled *BACE1-S* in the figure) through the stabilization of its mRNA. *BACE1-AS* is elevated in Alzheimer's disease, increasing the amount of BACE1 protein and, subsequently, the production of β-amyloid peptide. **d** | The role of the snoRNA in Prader–Willi syndrome (PWS). The loss of the snoRNA in PWS changes the alternative splicing of the serotonin receptor *HTR2C* precursor mRNA (pre-mRNA), resulting in a protein with reduced function. AβPP, amyloid-β precursor protein; CoREST, REST corepressor.

Cisplatin
A chemotherapy drug that is used to treat various types of cancers. It was the first member of a class of platinum-containing anti-cancer drugs, which now also includes carboplatin and oxaliplatin. These platinum complexes react *in vivo*, binding to and causing crosslinking of DNA, which ultimately triggers apoptosis (programmed cell death).

have also been implicated in cancer development; for example, PIWIL1 and PIWIL2 are overexpressed in a variety of somatic tumours[50–53]. Furthermore, studies in human cancer cell models have linked PIWIL1 overexpression to cell cycle arrest[54] and PIWIL2 overexpression to anti-apoptotic signalling and cell proliferation[55]. In a *D. melanogaster* model, ectopic expression of the genes encoding PIWI and other components of the piRNA machinery was shown to drive malignant brain tumour growth[56].

The mechanisms that underlie the putative oncogenic effects of piRNAs and PIWIs are largely unknown, although several pathways seem feasible. PIWI proteins are associated with stem cell self-renewal[57] and are re-expressed in precancerous stem cells that have the potential for malignant differentiation[58]. In some cancers, PIWIL2 overexpression has been suggested to lead to cells becoming resistant to cisplatin, which might arise because of increased chromatin condensation that prevents the normal process of DNA repair[59]. Strikingly, PIWI-associated RNAs were also found to be aberrantly expressed in human somatic tumours[60], implying that the PIWI pathway has a more profound function outside germline cells than was originally thought.

Table 2 | **Illustrative list of ncRNAs disrupted by either genetic or epigenetic means in cancer**

Name	Class	Disruption	Consequence	Cancer type
miR-124a	miRNA	CpG island hypermethylation	CDK6 overexpression	Colon, gastric, haematological
miR-34b and miR-34c	miRNA	CpG island hypermethylation	Metastasis	Many different tumour types
miR-148a	miRNA	CpG island hypermethylation	Metastasis	Colon, melanoma, breast
miR-9	miRNA	CpG island hypermethylation	Metastasis	Colon, melanoma, head and neck
miR-200c	miRNA	CpG island hypermethylation	EMT	Colon, breast, lung
miR-141	miRNA	CpG island hypermethylation	EMT	Colon, breast, lung
miR-205	miRNA	CpG island hypermethylation	EMT	Bladder
miR-196b	miRNA	CpG island hypermethylation	Unknown	Gastric
miR-129-2	miRNA	CpG island hypermethylation	SOX2 overexpression	Colon, endometrial, gastric
miR-137	miRNA	CpG island hypermethylation	CDC42 overexpression	Colon, head and neck
Uc.160+	T-UCR	CpG island hypermethylation	Unknown	Colon, breast, lung
Uc.283+A	T-UCR	CpG island hypermethylation	Cell survival, mitosis	Colon, breast, lung
Uc.346+	T-UCR	CpG island hypermethylation	Unknown	Colon, breast, lung
Uc.21	T-UCR	Mutation	Unknown	Epithelial tumours, leukaemia
Uc.72	T-UCR	Mutation	Unknown	Epithelial tumours, leukaemia
miR-151	miRNA	Gene amplification	Metastasis	Hepatocellular carcinoma
miR-517c and miR-520g	miRNA	Gene amplification	WNT upregulation	Neuroectodermal brain tumors
miR-106b-25	miRNA	Gene amplification	p21 and BIM depletion	Oesophageal adenocarcinoma
miR-15 and miR-16	miRNA	Genomic deletion	BCL2 overexpression	Haematological
U50	snoRNA	Genomic deletion	Increase growth	Breast
Uc.159	T-UCR	Genomic deletion	Unknown	Epithelial tumours, leukaemia

BCL2, B cell CLL/lymphoma 2; BIM, also known as BCL2L11; CDC42, cell-division cycle 42; CDK6, cyclin-dependent kinase 6; EMT, epithelial-to-mesenchymal transition; miRNA, microRNA; snoRNA, small nucleolar RNA; ncRNA, non-coding RNA; T-UCR, transcribed ultraconserved region.

snoRNAs. Insights into the potential roles of snoRNAs in cancer began with a study that reported substantial downregulation of snoRNAs in meningiomas compared with normal brains[61]. More recently, it was also shown that various snoRNAs are differentially expressed in non-small-cell lung cancer in comparison with the corresponding matched tissue[62]. Other studies have shown that a germline homozygous 2 bp (TT) deletion of the snoRNA U50 is associated with prostate cancer development[63] and that U50 undergoes frequent somatic heterozygous deletion and transcriptional downregulation in breast cancer[64]. Other studies showed that growth arrest specific 5 (*GAS5*) — a gene that hosts ten intronic snoRNAs but that also encodes an lncRNA — controls cell survival by inducing or sensitizing cells to apoptosis[65,66]. A substantial decrease of *GAS5* mRNA levels in breast cancer samples compared with adjacent unaffected normal breast epithelial tissues also suggests that it functions as a tumour-suppressor gene[65].

The association between snoRNAs and tumorigenesis also extends to their associated proteins. snoRNPs are divided into two main classes — C/D box and H/ACA box — according to their conserved secondary structural characteristics and associated modification reactions[67]. The C/D box snoRNPs consist of a core of four proteins — fibrillarin (the methyltransferase), nucleolar protein 56 (NOP56), NOP58 and NHP2-like 1 (NHP2L1) — whereas the H/ACA box snoRNPs contain dyskerin (the pseudouridine synthase), GAR1, NHP2 and NOP10. Fibrillarin is essential for development, and its depletion is lethal in embryos. Mutations in the human dyskerin (*DKC1*) gene, *NOP10* and *NHP2* are associated with the X-linked genetic disorder dyskeratosis congenita, one of the characteristics of which is susceptibility to epithelial cancers[68]. snoRNAs and snoRNPs are likely to contribute to tumorigenesis through an effect on ribosomes and protein translation, given that translation is often perturbed in cancer cells. However, snoRNAs might also be involved in the regulation of gene expression by giving rise to other regulatory RNA species, such as miRNAs[69]. Of particular interest for future research are the orphan snoRNAs, the functions and targets of which remain unknown.

lncRNAs. Recent findings suggest that levels of T-UCR transcription are altered in human tumorigenesis and that different types of human cancer can be distinguished according to their specific aberrant T-UCR expression profiles[33]. T-UCR expression signatures

have been described for chronic lymphocytic leukaemia (CLL), colorectal cancer (CRC) and hepatocellular carcinoma (HCC). Both downregulation and upregulation of different T-UCRs is seen when comparing expression in tumours and in normal tissues. Like miRNAs, T-UCRs that are differentially expressed in a specific cancer tend to be located in cancer-associated genomic regions (CARGs) that are associated with that type of cancer: for example, fragile sites, HOX gene clusters, minimal regions of loss of heterozygosity and minimal regions of amplification[33,70].

So far, the aberrant regulation of T-UCR expression in cancer has been found to occur in two main ways: by altered interactions with miRNAs[33] and by hypermethylation of CpG island promoters[34]. Many T-UCRs show significant complementarity to specific miRNAs, which suggests that the T-UCRs could act as miRNA targets[31,71]. Experimental results support this hypothesis. For instance, transfection of miR-155 into leukaemia cells significantly reduces expression of the T-UCR uc.160+ (REF. 33). As occurs in coding genes, DNA hypermethylation of a CpG island that is located in the promoter can also downregulate T-UCR expression[34]. This has been shown for several T-UCRs in a manner that is specific to cancer type. Finally, recent reports also show that germline point mutations (for example, in uc.21 and uc.72) and deletions (for example, in uc.159) in UCRs are about three times more frequent in CLL and CRC patients than in controls[72].

Given the evidence above, great efforts have been made to characterize the roles of T-UCRs in cancer, but little is known about their biogenesis and normal functions. The T-UCR uc.338 seems to modulate cell growth in human hepatocytes: its downregulation in HepG2 cells reduces the length of S phase and increases the sub-G1 peak[73], whereas the reintroduction of uc.283+ in HCT-116 cells increases cell-doubling time and cell death[34].

Among various examples of the involvement of lncRNAs in cancer, the role of *HOTAIR* in human neoplasia is the most well-understood[24]. In epithelial cancer cells, *HOTAIR* overexpression causes polycomb to be retargeted across the genome (FIG. 2). The invasiveness of these cells and propensity to metastasize is also increased in these cells, both of which changes are dependent on the polycomb protein PRC2. By contrast, cancer invasiveness is decreased when *HOTAIR* expression is lost, an effect that is increased in cells with higher than usual levels of PRC2 activity. As such, *HOTAIR* might have an active role in modulating the cancer epigenome and mediating cell transformation. A similar function has been postulated for some other lincRNAs, such as lincRNA-p21, which functions as a repressor in p53-dependent transcriptional responses[28]. The p15 antisense lncRNA, *p15AS*, which was first identified in human leukaemia, has also been shown to induce the silencing of the p15 tumour suppressor gene locus by inducing the formation of heterochromatin[74].

Disruption of ncRNAs in other diseases

We have only just lifted the lid of the Pandora's box that is the contribution of ncRNA disruption in other human diseases. Just as in cancer, miRNAs have been the first ncRNAs for which roles in other diseases have been elucidated. As with the disrupted miRNA expression patterns that were observed in transformed cells, neurodegenerative, inflammatory and cardiovascular diseases show aberrant miRNA transcription signatures (TABLE 3). Insights into the roles of other ncRNAs in non-neoplastic disease are just beginning to emerge.

miRNAs in neurological disorders. Among their varied roles, many miRNAs are essential for the correct function of the nervous system, which has the broadest spectrum of miRNA expression of all human tissues. Around 70% of miRNAs are expressed in brain, and many of them are specific to neurons[75]. They are involved in neurodevelopment, dendritic spine formation and neurite outgrowth, and their dysregulation has been described in almost all neurological diseases studied.

In mammals, conditional ablation of essential components of the miRNA machinery, such as Dicer, suggests that miRNAs are crucial for brain function. For instance, the importance of miRNAs for terminal differentiation and the maintenance of many neuronal types has been demonstrated[76]. Disruption of miRNA processing has been shown to cause hallmarks of: ataxia in Purkinje cells[77]; multiple sclerosis in oligodendrocyte cells[78]; Parkinson's disease in dopaminergic cells[76]; and Alzheimer's disease in α-calcium/calmodulin-dependent protein kinase type II (αCaMKII)-expressing neurons[79]. Mutations in the central miRNA-processing machinery are also consistently found to be causal in cases of various neurological disorders. For example, mutations in components of the Drosha microprocessor complexes, such as Fus and TDP-43, cause up to 50% of familiar amyotrophic lateral sclerosis[80]; fragile X syndrome (FXS) can be caused by mutations in the RISC complex component fragile X mental retardation 1 protein (FMRP)[81], and translational repression by miRNAs is negatively regulated by mutations in leucine-rich repeat serine/threonine-protein kinase 2 (LRRK2), which is a cause of Parkinson's disease[82]. In addition, widespread miRNA dysregulation has been described in many neurological disorders. A few causes of this dysregulation have been characterized, such as the alteration in miRNA dosage that occurs as a consequence of trisomy 21 in Down's syndrome[83] and disease-associated SNPs at miRNA loci in multiple sclerosis, Alzheimer's disease and Parkinson's disease[84,85].

Specific miRNAs have also been linked to particular neurological diseases. Examples include miR-206 deficiency that accelerates amyotrophic lateral sclerosis[86], miR-9 defects in spinal motor neuron disease[87] and miR-19, miR-101 and miR-130 levels, which modify the penetrance of spinocerebellar ataxia type 1 by the co-regulation of ataxin 1 levels[88]. In general, however, from a functional standpoint, the validation of the role of miRNA dysregulation in neurological diseases strongly depends on how much is known about the molecular basis of disease pathology — this can determine whether the specific target genes or pathways of the miRNAs in question can be elucidated. For example, in Alzheimer's

Loss of heterozygosity
A loss of one of the alleles at a given locus as a result of a genomic change, such as mitotic deletion, gene conversion or chromosome mis-segregation.

CpG island
A genomic region enriched for CpG dinucleotides that often occurs near constitutively active promoters. Mammalian genomes are otherwise depleted of CpGs owing to the preferential deamination of methylated cytosines.

Sub-G1 peak
On cell staining with a DNA-intercalating dye such as propidium iodide, a DNA profile representing cells in G1, S phase and G2M will be observed with apoptotic cells being represented by a sub-G0/G1 population seen to the left of the G0/G1 peak.

Dendritic spine
A mushroom-shaped structure on neuronal dendrites that receives synaptic input and has postsynaptic densities. Changes in spine shape are thought to be important for modulating synaptic strength.

Ataxia
Inability to coordinate movement.

Penetrance
The proportion of individuals with a specific genotype who manifest the genotype at the phenotypic level. If penetrance of a disease allele is 100% then all individuals carrying that allele will express the associated disorder and the genotype is said to be 'completely penetrant'.

Table 3 | **Illustrative list of ncRNAs that are disrupted in non-tumoural disorders**

Disease	Involved ncRNAs	ncRNA type	Refs
Spinal motor neuron disease	miR-9	miRNA	87
Spinocerebellar ataxia type 1	miR-19, miR-101, miR-100	miRNA	88
Amyotropic lateral sclerosis	miR-206	miRNA	86
Arrhytmia and hypertension	miR-1	miRNA	98
Atheromatosis and atherosclerosis	miR-10a, miR-145, mR-143 and miR-126	miRNA	100–102
Atheromatosis and atherosclerosis	Circular ncRNA linked to the CDKN2A locus	lncRNA	119
Cardiac hypertrophy	miR-21	miRNA	144
Rett's syndrome	miR-146a, miR-146b, miR-29 and miR-382	miRNA	108,109
5q syndrome	miR-145 and miR-146a	miRNA	106
ICF syndrome	miR-34b, miR-34c, miR-99b, let-7e and miR-125a	miRNA	107
Crohn's disease	miR-196	miRNA	110
Prader–Willi and Angelman syndromes	snoRNA cluster at 15q11–q13 imprinted locus	snoRNA	114–116
Beckwith–Wiedeman syndrome	lncRNAs *H19* and *KCNQ1OT1*	lncRNA	145
Uniparental disomy 14	snoRNA cluster at 14q32.2 imprinted locus	snoRNA	145
Silver–Russell syndrome	lncRNA *H19*	lncRNA	145
Silver–Russell syndrome	miR-675	miRNA	145
McCune–Albright syndrome	lncRNA *NESP-AS*	lncRNA	145
Deafness	miR-96	miRNA	111
Alzheimer's disease	miR-29, miR-146 and miR-107	miRNA	89–91
Alzheimer's disease	ncRNA antisense transcript for *BACE1*	lncRNA	112
Parkinson's disease	miR-7, miR-184 and let-7	miRNA	82
Down's syndrome	mir-155 and miR-802	miRNA	83
Idiopathic neurodevelopmental disease	T-UCRs uc.195, uc.392, uc.46 and uc.222	T-UCR	113
Rheumatoid arthritis	miR-146a	miRNA	147
Transient neonatal diabetes mellitus	lncRNA *HYMAI*	lncRNA	148
Pseudohypoparathyroidism	lncRNA *NESP-AS*	lncRNA	146

BACE1, β-secretase 1; CDKN2A, cyclin-dependent kinase inhibitor 2A; *HYMAI*, hydatidiform mole associated and imprinted; ICF syndrome, immunodeficiency, centromeric region instability and facial anomalies syndrome; KCNQ1OT1, KCNQ1 opposite strand/antisense transcript 1; lncRNA, long non-coding; miRNA, microRNA; NESP, also known as GNAS; NESP-AS, NESP antisense; ncRNA, non-coding RNA; snoRNA, small nucleolar RNA; T-UCR, transcribed ultraconserved region.

disease, abnormally expressed miRNAs have consistently been reported as downregulating the expression of the enzyme β-secretase 1 (BACE1), which leads to the production of β-amyloid peptide[89–91]. miRNAs are also known to control the inflammatory process that leads to the development of multiple sclerosis[92,93]. Finally, miRNAs regulate α-synuclein expression[94] and E2F1 and dopamine production[82]; thus, their aberrant expression might underlie Parkinson's disease.

miRNAs in cardiovascular disorders. Several lines of evidence suggest key roles for miRNAs in cardiovascular disorders. Tissue-specific deletion of Dicer in mice causes lethal phenotypes in myocardial and vascular lineages[95,96]. The most abundant miRNA in cardiac myocytes is miR-1, which is involved in heart development[97]. This miRNA has been linked with the development of arrhythmias as it downregulates expression of the ion channel genes[98]. Furthermore, myocardial tissue from heart failure patients has a distinct miRNA expression profile as compared to a healthy myocardium[99]. miRNAs also have a role in vascular disease. Here, the formation of the atheroma plaque and the physiological phenotype of vascular smooth muscle cells depends on the correct expression of several miRNAs, such as miR-10a, miR-145, miR-143 and miR-126[100–102]. As with heart failure, an miRNA expression signature distinguishes the vascular lesion from healthy tissue[103]. Finally, SNPs that affect miRNA-binding sites, including those for miR-1, have been associated with unrestricted muscular growth[104] and hypertension[105], which increase the risk of cardiovascular disease.

miRNAs in other diseases. Several monogenic disorders have been found to have aberrant miRNA expression profiles in tissue types that are relevant to the pathophysiology of the disease, and this list is rapidly increasing. In addition, specific miRNA defects have been shown to underlie particular diseases; for example, miR-145 and miR-146a deletions are involved in the 5q syndrome phenotype[106]. As well as genetic mutations that underlie miRNA dysfunction in inherited disease, we should

5q syndrome
A rare disorder caused by loss of part of the long arm (q arm, band 5q31.1) of human chromosome 5.

also consider those diseases in which alterations of epigenetic marks cause misregulation of ncRNA expression. Recent disease studies have found disrupted expression of miRNAs that are transcribed from CpG islands, at which the expression of the miRNA is regulated by DNA methyltransferases (DNMTs). This situation occurs in immunodeficiency, centromere instability and facial anomalies (ICF) syndrome, in which patients harbour mutations in the gene that encodes DNMT3B[107], and in Rett's syndrome, wherein mutations in the methyl CpG-binding protein 2 (*MECP2*) gene are responsible[108,109]. Dysregulation of miRNAs in ICF syndrome has also been associated with an aberrant histone modification profile[107].

An ever-growing number of miRNAs are being linked to the development of many other human diseases. For example, the presence of altered binding sites for miRNAs has been described in inflammatory colorectal Crohn's disease[110], and a mutation in miR-96 has been related to deafness[111]. It is likely that the examples described above are just the tip of the iceberg.

Other ncRNAs in non-neoplastic disorders. Other types of ncRNA have been implicated in non-neoplastic disease. For example, in Alzheimer's disease, a conserved non-coding antisense transcript drives rapid feed-forward regulation of BACE1 (REF. 112) (FIG. 2). In addition, enrichment for T-UCR loci is seen in chromosomally imbalanced regions that are associated with pathology in neurodevelopmental disorders[113]. snoRNAs have been found to have an important role in imprinting disorders, specifically those with a neurodevelopmental component such as Prader–Willi syndrome (PWS) and Angelman syndrome[114]. These disorders are caused by several genetic and epigenetic mechanisms involving the 15q11–q13 imprinted locus, which contains a cluster of tandemly arranged snoRNAs[115,116]. The loss of one of these snoRNAs, *HBII-52*, has been implicated in PWS, in which it changes the alternative splicing of the serotonin receptor HTR2C precursor mRNA (pre-mRNA)[115] (FIG. 2).

Potential links between other ncRNAs and disease are intriguing but are currently more tentative. Knockout mouse models for the proteins that are involved in piRNA biogenesis (MIWI1, MILI2 and MIWI23) revealed a profound reactivation of transposon activity, which is thought to be responsible for the observed sterility in this mouse owing to impaired spermatogenesis[17,117]. Interestingly, in humans, a SNP in the 3′UTR of PIWIL4 was associated with an increased risk of spermatogenic failure[118]. Finally, an enhanced risk of atherosclerosis has been associated with the expression of a non-polyadenylated circular ncRNA that is linked to the cyclin-dependent kinase inhibitor 2A (CDKN2A; also known as INK4/ARF) locus[119].

Therapies targeting ncRNAs

ncRNAs and the protein machineries that are involved in their biogenesis or activity have become targets of novel therapeutic approaches (FIG. 3). So far, most work in this area has been in the context of the role of miRNAs in cancer[120]. Although it is early days, there is also considerable interest in applying similar approaches to other types of disease, both for miRNAs and for other ncRNAs.

Therapies that inhibit miRNA function. The knowledge that miRNAs regulate their targets through base pairing has led to the use of antisense oligonucleotides (ASOs) to inhibit miRNA function therapeutically. ASOs inhibit miRNA targets based on base pair complementarity. Three main classes of ASOs that have been developed are locked nucleic acids (LNAs), anti-miRNA oligonucleotides (AMOs) and antagomirs that incorporate different chemical modifications to increase stability and efficacy[121–123].

The efficacy of ASOs has been demonstrated in some cases; for example, in a mouse mammary tumour model, the intravenous injection of antagomirs targeting miR-10b prevents the onset of metastasis, suppressing dissemination to the lungs[124]. In the context of cardiovascular disease, LNAs have been targeted against miR-122, an miRNA that is involved in regulating cholesterol and lipid metabolism. Systemic administration of these LNAs has been shown to antagonize the function of miR-122 in the liver and to decrease plasma cholesterol levels, without evidence of toxicity, in a manner that is dependent on the dose of the LNAs[122].

However, silencing of a single miRNA might not be sufficient in all cases owing to the pleiotropic and multifaceted biology of cancer cells. Recent research in this field suggests that several miRNAs can be simultaneously inhibited using single ASOs that are targeted against multiple miRNAs. In this approach, multiple antisense units are engineered into a single unit to produce what the authors termed a multiple-target anti-miRNA antisense oligodeoxyribonucleotide (MTg-AMO)[125]. One MTg-AMO was designed to target miR-21, miR-155 and miR-17-5p, which are three oncogenic miRNAs that are overexpressed in many tumours. Use of this MTg-AMO resulted in an increased inhibition of cancer growth compared with both individual AMOs that are targeted against a single miRNA and combinations of such single-target AMOs[125]. In the future, MTg-AMOs could be designed to simultaneously inhibit the function of miRNAs that have roles in driving several key aspects of cancer biology: evasion of apoptosis, unchecked cell division, angiogenesis, tissue invasion and metastasis.

Another innovative strategy involves expressing competitive inhibitors of miRNA function. These 'miRNA sponges' are vectors containing multiple artificial miRNA binding sites that are placed under the control of strong promoters to produce large quantities of transcript. They act as sponges for cognate miRNAs, preventing their association with natural targets[126,127]. This strategy has been used, for example, to inhibit miR-9 in highly malignant cells, demonstrating the role of miR-9 in metastasis[127].

Therapies that restore miRNA function. Several strategies have been suggested to restore the function of miRNAs with tumour suppressor properties that are

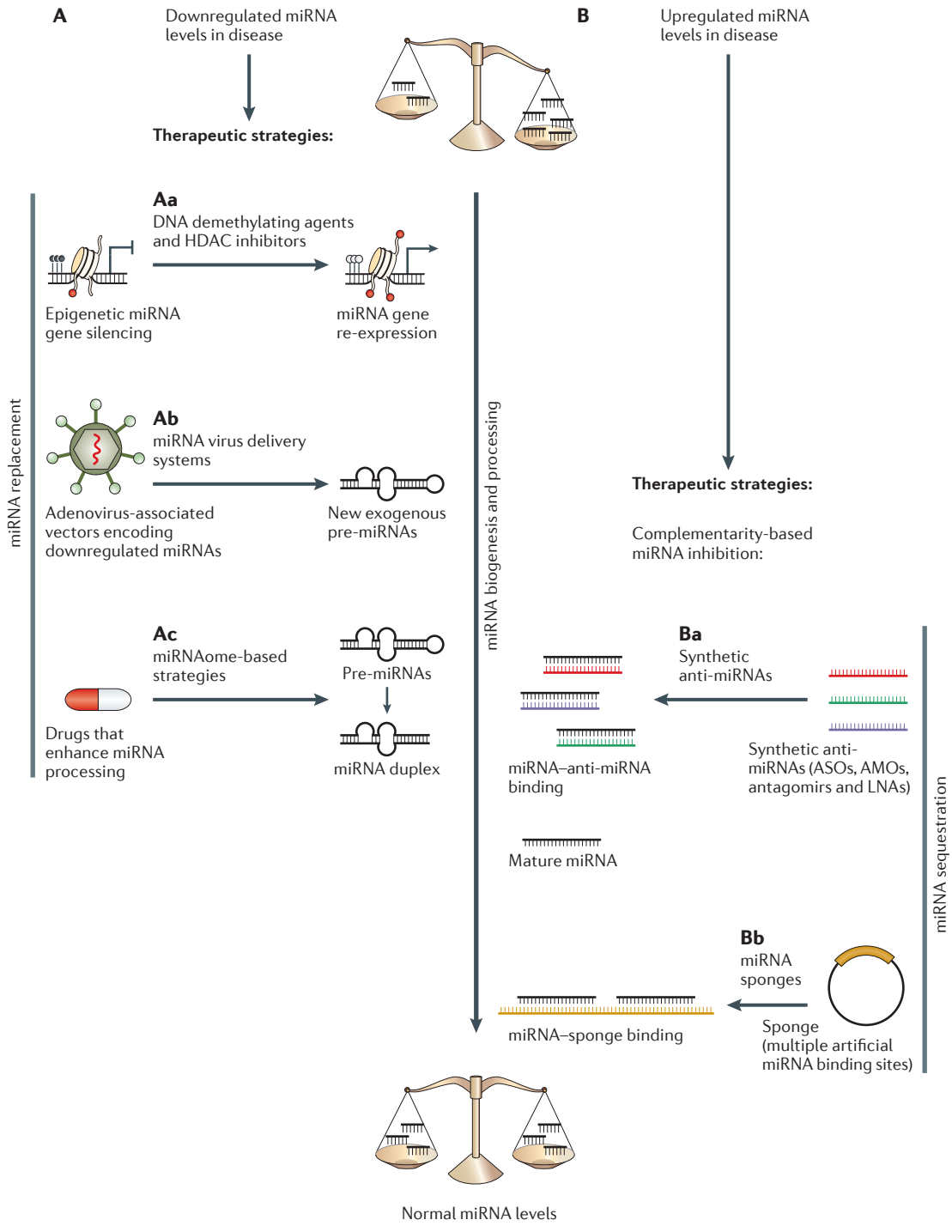

Figure 3 | **Therapies targeting miRNAs in human disease.** Aberrant microRNA (miRNA) levels are associated with multiple diseases. **A** | Strategies to increase miRNA levels include the following. **Aa** | Epigenetic drug treatments for reactivating the transcription of silenced miRNA genes, using DNA demethylating agents and histone deacetylase (HDAC) inhibitors. **Ab** | Replacement of miRNAs using virus delivery systems: for example, adenovirus-associated vectors (AVV) containing genes that code for downregulated miRNAs. **Ac** | miRNAome-based strategies based on drugs such as enoxacin, which enhance miRNA production by binding to TAR RNA-binding protein 2 (TARBP2), an integral component of a DICER1-containing miRNA-processing complex. **B** | Conversely, upregulated miRNAs can be sequestered by means of therapies based on base-pair complementarity, which include the following. **Ba** | Synthetic antisense oligonucleotides (ASOs) or modified ASOs that incorporate chemical groups to improve the stability and efficacy, such as anti-miRNA oligonucleotides (AMOs), antagomirs or locked nucleic acids (LNAs). **Bb** | miRNA sponges: vectors that contain multiple artificial miRNA binding sites that act as sponges for the cognate miRNA, preventing its association with endogenous targets.

downregulated in cancer. A recent study reported the use of a strategy named 'miRNA replacement therapy', which aims to restore miR-26a expression in hepatocellular carcinoma. An adenoviral vector was used to deliver this miRNA in a mouse model of hepatocellular carcinoma, resulting in suppression of proliferation and induction of apoptosis, thereby inhibiting cancer progression[128]. However, viral delivery of miRNAs presents the same challenges as those presented by the delivery of protein-coding genes in more traditional gene therapy strategies[129].

A large body of evidence shows that most human tumours are characterized by defects in miRNA production that lead to global miRNA downregulation. It is therefore tempting to speculate that restoring the global miRNAome could have a therapeutic effect. Global miRNA repression triggers cellular transformation and tumorigenesis in both *in vitro* and *in vivo* models[43,45,130]. As a result of these findings, a new 'miRNAome-based' strategy has been suggested. The small-molecule drug enoxacin enhances RNAi and promotes miRNA processing by binding to TARBP2 (REF. 131). Proof-of-principle studies in human cancer cell lines and xenografted primary tumours have shown that, through the global reconstitution of downregulated miRNAs to a more 'normal' miRNA expression pattern following enoxacin treatment, the malignant phenotype can be blocked[132]. The drug did not affect normal cells and was not associated with toxicity in mouse models[132].

Finally, another approach for restoring the global miRNAome is the use of DNA demethylating agents and histone deacetylase inhibitors. These compounds release the epigenetic silencing of tumour suppressor ncRNAs, as has been shown for miRNAs and T-UCRs, thereby stopping tumoural growth and ultimately resulting in the programmed cell death of the transformed cells[34,133,134]. These agents, even without any target specificity, have shown themselves to have therapeutic benefits and have received clinical approval for the treatment of certain haematological malignancies[135].

Targeting other types of ncRNAs. Similar approaches to those described above for inhibiting the function of deregulated miRNAs could be useful for other ncRNAs, thereby increasing the number of therapeutic targets. Successful inhibition of long ncRNAs seems to be more difficult than with miRNAs, presumably owing to their extensive secondary structures, although knockdown of lincRNAs using siRNAs can be achieved[24,136]. The complexity of lincRNAs means that new bioinformatic tools are required in order to design inhibitors. For example, methods such as systematic evolution of ligands by exponential enrichment (SELEX) have been proposed to identify RNA sequences that bind to lincRNAs[137]. This strategy has already been used to identify RNA sequences that bind to primary miRNAs (pri-miRNAs; see FIG. 1)[138].

Our growing knowledge of other ncRNAs is also being exploited to develop new therapeutic strategies not only against cancer but also for other conditions. As mentioned earlier in this Review, a non-coding antisense transcript regulates BACE1, a crucial enzyme in Alzheimer's disease[112]. This finding and others are attracting the attention of the pharmaceutical and biotechnology industries. Companies and institutions such as the Allen Institute for Brain Science, CuRNA, Regulus Therapeutics, Miragen Therapeutics and Santaris Pharma are developing ncRNA-based strategies against cancer, cardiovascular, neurological and muscular diseases[139]. Although further studies are required, the results obtained so far in cancer cell lines, mouse models and non-human primates are promising. It is to be hoped that the potential benefits of ncRNA-based therapies will be translated into clinical practice in the near future.

Future perspectives

Interest in the contribution of ncRNAs to the genesis and progression of human disorders is booming, but much effort is now required to determine the full extent of this contribution and the mechanisms by which ncRNAs exert their pathological effects.

One important challenge will be to identify all functional ncRNAs that are encoded in the human genome, and emerging genomic, epigenomic and bioinformatic approaches will be crucial in this context. Efforts such as the Encyclopedia of DNA Elements (ENCODE) project — which aims to identify all functional elements in the human genome[140] — are making important headway. Methods based on second-generation sequencing, such as RNA sequencing, will provide a more detailed picture of the whole human ncRNA transcriptome. Bioinformatic tools for identifying potentially functional ncRNAs will also be important. Because ncRNAs fold into complex secondary structures that are crucial to function, sequence-based alignments alone might not be enough to identify ncRNAs. A number of algorithms that use different approaches have been developed to identify potentially functional ncRNAs (for example, RNAfold, RNAalifold, Pfold, EvoFold, RNAz, QRNA, CMFinder and FOLDALIGN). However, so far, only a few kinds of ncRNA can be identified using these programs, including rRNAs, tRNAs, snoRNAs and miRNAs. In general, several issues make the identification of functional ncRNAs and the assignment of a particular function challenging: for example, the lack of a complete understanding of functional motifs or domains in ncRNAs, the low expression levels of some ncRNAs, and the need for a better definition of their regulatory regions. However, the restricted spatio-temporal expression of many ncRNAs, as well as the binding of transcription factors to non-coding loci[2], could be used as evidence of functionality. Furthermore, the purifying selection that acts on ncRNA promoters[141] suggests a strictly regulated expression and function that is not expected for transcriptional 'noise' and can therefore be used to infer functionality of an ncRNA.

Specifically in relation to disease, understanding the precise roles of ncRNAs, particularly non-miRNAs, is a key challenge. The development of mice models for single ncRNAs have been useful in relating the altered expression of some of these transcripts to particular human disorders. A good example is a mouse model

Systematic evolution of ligands by exponential enrichment
(SELEX). A set of laboratory procedures that are generally used for the identification of representative sets of ligands for a protein. In the context of RNA, this is a method for identifying consensus-binding sequences on RNA substrates by *in vitro* selection of short RNAs that bind preferentially to RNA-binding proteins.

Second-generation sequencing
Used in this Review to refer to sequencing methods that have emerged since 2005 that produce millions of typically short sequence reads (50–400 bases) from amplified DNA clones. It is also often known as next-generation sequencing.

RNA sequencing
An experimental protocol that uses next-generation sequencing technologies to sequence the RNA molecules within a biological sample in an effort to determine the primary sequence and relative abundance of each RNA.

that conditionally expresses miR-21, which revealed the function of this miRNA as a genuine oncogene[142]. Similar models could be used to study the potential oncogenic role of other candidates, such as miRNA-372 and miRNA-373[143]. Biochemical and biological tools, such as antagomirs, have been useful in elucidating the function of miRNAs, and similar tools will be important in studying the function of other ncRNAs. Improved cloning techniques and better approaches for mapping TSSs are also needed to characterize ncRNAs so that these ncRNAs can be studied by overexpression and promoter regulation. For example, rapid amplification of cDNA ends (RACE) methodology has been used to define the origin of transcription of T-UCRs[34], but the process is very time consuming. The complete cloning of the full-length transcripts of these ncRNAs might provide further functional clues, along with knock-in and knockout experiments in cellular and animal models.

Finally, expectations in the therapeutic arena have been raised as a result of the recognition of ncRNA defects in human diseases. Molecules based on ncRNAs broaden the universe of potential 'druggable' targets, and important cancer-promoting genes (such as *MYC* and other transcription factors) that are not considered to be viable conventional drug targets can now be readily inhibited by siRNAs or miRNAs. The first clinical trials using ncRNA-based molecules are underway. However, new approaches are imminent, such as small molecule drugs that target the miRNA machinery. Although there is a long way to go to establish genuine treatments using these latter agents, these approaches might be useful for overcoming a key problem that is faced by traditional cancer therapy: the development of resistance. Drug resistance through mutation of target gene sequences could be a problem for miRNA strategies, just as it is for conventional drugs. However, it would presumably be much more difficult for a cancer cell to resist the actions of drugs that, by targeting the cell's miRNAome as a whole, aim to revert the transformed cell to a more 'normal-like' ncRNA expression profile. The scientific community and pharmaceutical companies must strive harder in pursuit of these new approaches (by using automated large-scale screening of these miRNA-related drugs, developing knock-in and knockout models for the target ncRNAs, and so on). The targeting of other ncRNAs and other human diseases, in addition to miRNAs and cancer, is still in its infancy, but new important developments are expected in this area. Exciting times lie ahead for us.

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