Discuss recent advances in our understanding of the pathobiology of non-coding RNAs

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Introduction

The postulation of the 'Central Dogma' by Francis Crick apparently solved the mystery surrounding the function of ribonucleic acid (RNA) in the early days of molecular biology(1). This classical paradigm highlights the role of RNA as an intermediate product of gene expression between DNA and functional proteins. However, more recent evidence has suggested that this is a gross oversimplification. It is now recognised that besides messenger RNA (mRNA), a diverse range of RNA molecules exist within the cell. In particular, an increasing body of evidence indicates that non-coding RNAs (ncRNAs) are key components of both human physiology and pathology, and responsible for orchestrating a plethora of cellular functions – many of which we are only beginning to unravel. Thus more than half a century since Crick first stated his 'Central Dogma', the full spectrum of RNA functioning remains as elusive as ever.

The aim of the present essay is to discuss recent advances in our understanding of the pathobiology underlying this fascinating group of molecules.

Venturing beyond the gene: an expanding RNA universe

The essential cellular roles of some non-translated 'housekeeping' RNAs such as ribosomal and transfer RNAs have been known since the 1950s(3). However, the traditional view that proteins alone underpin the biological phenotype in eukaryotes largely persisted until the discovery of the first known microRNA (miRNA) in 1993(4). MiRNAs are a type of short ncRNAs, and greater efforts were subsequently directed at exploring the field of RNAs with little or no protein-coding potential. More recently, the interest into ncRNAs has been further catalysed by the rapid rise in large-scale genomic sequencing, which has revealed a surprisingly small number of protein-coding genes in

humans. Surely, these 20,000 or so genes, representing <2% of the total genomic sequence(5), cannot be solely responsible for our developmental and physiological complexity? Indeed, humans have virtually the same number of protein-coding genes as significantly less complex eukaryotes such as the nematode *Caenorhabditis elegans*!

Subsequently, it has become apparent that the number of non-coding sequences correlates with biological complexity(6). Furthermore, data from recent high-throughput transcriptomic analyses such as FANTOM(7) and ENCODE(8) demonstrate that up to 90% of our genomic DNA is transcribed, with the vast majority being so-called long ncRNAs (lncRNAs)(9). These unexpected findings indicate that long non-coding transcripts constitute a substantial proportion of the total mammalian RNA population. Indeed, the known repertoire of lncRNAs continues to grow at breakneck speed; with almost 60,000 human lncRNAs reported to date(10), we are undoubtedly entering an era of '*lncRNAomics'*.

Long non-coding RNAs

NcRNAs may have dramatically redefined the traditional paradigm of mammalian genomic organisation, but the term 'long non-coding RNA' has still eluded comprehensive definition. One group has rather conservatively defined lncRNAs as *'RNA molecules that may function as either primary or spliced transcripts and do not fit into known classes of small RNAs or into classes of structural RNAs'*(11). The need for caution is wise, as aside from the absence of a translated open reading frame, many lncRNAs are virtually indistinguishable from mRNAs in terms of biogenesis and biochemistry(12). Additionally, the 'typical' length of an lncRNA is still debated. In order to partition them from short ncRNAs, lncRNAs are commonly defined as transcripts greater than 200 nucleotides, which can be further classified into five subclasses depending on their proximity to the closest coding genes(13). Unfortunately, this method of categorisation is far from comprehensive, with 200 nucleotides being an arbitrary cut-off that corresponds to the sensitivity threshold of contemporary RNA extraction techniques. LncRNAs are in fact highly heterogeneous in size, with some spanning 100 kilobases(14). Furthermore, with the onset of more sensitive techniques such as

mass spectrometry proteomics, a small number of lncRNAs have been found to actually encode micropeptides, thus rendering the definition 'non-coding' a misnomer(15).

From a functional perspective, attempts to shed light upon this previously unexplored 'dark matter' of the genome has been far from straightforward. In direct contrast to miRNAs, whose roles in transcriptional and post-transcriptional gene silencing are comparably well-documented(16), only a small fraction of lncRNAs identified to date has been fully characterised. As such, the overall importance and precise functions of lncRNAs remain unclear. Considering their relatively poor primary sequence conservation(17), relative instability(18), and almost complete lack of protein-coding potential, it has been suggested that lncRNAs may merely be evolutionary 'debris' resulting from random, meaningless transcription of inert sequences – sometimes referred to as 'transcriptional noise'(19). However, emerging evidence has supported the notion of lncRNAs representing a 'second genetic code', therefore being critical to normal cellular functioning. The current consensus is that many lncRNAs represent key players in the regulation of diverse physiological processes including cell differentiation, development, signalling, and metabolism(20).

Long non-coding RNAs and cancer

Of particular relevance to the contemporary pathologist, lncRNAs have been implicated in a growing number of human diseases. In particular, their association with cancer has eclipsed that of any other pathological condition(21). Recently, numerous lncRNAs have been systematically identified in multiple cancer transcriptomes, establishing their association with the majority of cancer types(22)**[Figure 1]**. A role for lncRNAs in cancer is perhaps unsurprising given their importance

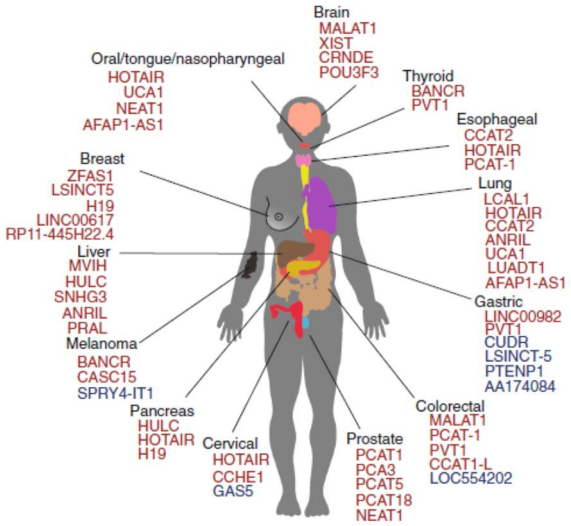

Figure 1. Overview of some lncRNAs known to associate with specific cancers. As can be seen, lncRNAs have been implicated in virtually every major cancer type. Taken from (2).

in cellular homeostasis, and the strong link between cancer and genomic perturbations. Indeed, a number of short ncRNAs have also been implicated, including miRNAs, piRNAs, and snoRNAs. MiRNAs remain arguably the most well-studied ncRNA class in cancer to date, with their role in tumorigenesis first identified in 2002(23). Nonetheless, the interest within the scientific community for lncRNA involvement in cancer pathogenesis has seen an exponential rise in recent years**[Figure 2]**.

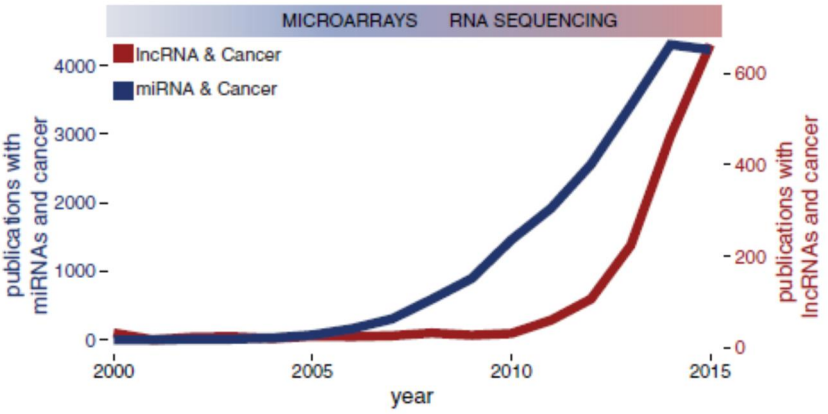

Figure 2. A comparison of the interest within the science community for involvement of lncRNAs (red) and miRNAs (blue) in cancer as measured by the number of relevant publications over time. Data (2016 excluded) was collected using a PubMed search for 'lncRNA cancer' or 'miRNA cancer'. While the number of studies on miRNAs and cancer has been gradually increasing since 2005, this has recently plateaued and set soon to be leapfrogged by the dramatic recent rise in efforts dedicated to uncovering the role of lncRNAs in cancer. Taken from (2).

Dysregulated expression of lncRNAs in cancer

An important clue into the pathological significance of lncRNAs stemmed from studying the cellular expression profiles of single transcripts. Under normal conditions, many lncRNAs display remarkable cellular specificity(24) and a relatively restricted expression pattern within the cytoplasm and nucleus(25), suggesting an extremely precise regulation of their transcription and activity. However, techniques such as microarray, and more recently RNA sequencing and real-time PCR, have revealed differential lncRNA expression in a wide array of tumour types. To use just one example, levels of the HOX Antisense Intergenic RNA (HOTAIR) have been found to be elevated in breast and various gastrointestinal cancers(26)**[Table 1]**. It is likely that with the onset of novel RNA sequencing techniques such as *CaptureSeq*, even rare or poorly expressed transcripts associated with cancer can be identified in the near future(27).

Drivers of aberrant lncRNA expression

Genomic alterations

A number of potential causes have been proposed to induce altered lncRNA expression. These include epigenetic changes such as loss of imprinting(28), in addition to genomic alterations including mutations, deletions, and amplifications. Given that 43% of disease- or trait-associated single nucleotide polymorphisms (SNPs) are found outside of protein-coding genes(29), it is likely that a large proportion of lncRNAs lies within or close to fragile sites such as common breakpoints and SNPs. This suggests that the primary sequences of at least some lncRNAs would be particular susceptible to damage. In support of this, genome-wide association studies have revealed the presence of lncRNAs in specific cancer types based on their overlap with cancer risk loci. This is illustrated by the identification of the lncRNAs CASC15 and NBAT1 as part of the 6p22 locus; the latter contains SNP rs6939340, which is linked to neuroblastoma progression(30). The precise mechanism by which small mutations such as SNPs lead to altered lncRNA expression is unclear, but SNPs located in genomic regions involved in transcriptional control could potentially interfere with promoters and enhancers within the lncRNA gene, leading to altered levels of transcript production.

Mutations in oncogenic transcription factors

An additional driver for altered lncRNA expression in cancer takes the form of mutations or abnormal expression of transcription factors that are known to interact with lncRNAs. In a murine model of gallbladder cancer, ectopic expression of the canonical oncogene c-Myc has been demonstrated to induce HOTAIR expression via promoter binding, with gene knockdown exerting the opposite effect(31). To add on a further layer of complexity, lncRNA expression in tumour cells is altered differentially depending on whether they are part of an oncogenic or tumour suppressor signalling network. Thus while HOTAIR, being a pseudo-oncogenic lncRNA, is overexpressed in transformed cells(32), levels of MEG3, which is involved in the p53 transcriptional cascade, are reduced(33). Interestingly, some lncRNAs such as H19 and Xist can behave as either oncogenes or tumour suppressors depending on the cellular targets present, and the levels of these transcripts therefore vary between tumour types**[Table 1]**.

LncRNA	Cancer type	Up/down-regulated	Oncogenic/tumour suppressor activity	Main mechanism of action
HOTAIR	Breast, colorectal, gallbladder, liver & gastric	Upregulated	Oncogenic	Chromatin remodelling
MEG3	NSCLCs & pituitary adenomas/meningiomas	Downregulated	Tumour suppressor	Regulation of p53 transcriptional cascade
XIST	Haematological & breast	Up- or downregulated depending on cancer type	Both	miRNA 'decoy'
H19	Bladder, lung, liver & breast	Up- or downregulated depending on cancer type	Both	Control of imprinting

Table 1. Examples of several lncRNAs associated with cancer. These transcripts could either be up-or downregulated depending on whether they belong to an oncogenic or tumour suppressor signalling network. The main mechanism of action for each lncRNA is shown, although it must be emphasised that individual lncRNAs typically display multiple modes of action.

Mechanisms of lncRNA dysfunction in cancer

The involvement of lncRNAs in tumorigenic signalling pathways has important pathological implications. It suggests that perturbed lncRNA expression in tumours has direct functional consequences, thus influencing disease phenotype. Importantly, functional assessments using RNA interference and modified antisense oligonucleotide strategies have demonstrated a role of lncRNA dysfunction in virtually all the characteristic hallmarks of cancer such as promotion of angiogenesis and metastatic spread(34). What makes this possible? An elegant array of mechanisms by which lncRNAs normally carry out their functions at the molecular level has been elucidated(35), and many of these can potentially be involved in driving the cancer phenotype(2). HOTAIR represents one of the most thoroughly investigated lncRNAs to date(26) and its role in facilitating invasion and promoting metastasis in breast cancer will be used to illustrate several of these pathobiological mechanisms**[Figure 3]**.

HOTAIR as an exemplary inducer of breast cancer metastasis

Structural scaffold for PRC2 and BRCA1

Although proteins are often regarded as the primary conductors of scaffolding complexes, as highlighted by the abundance of A-kinase anchoring proteins within the cell, emerging evidence suggests that particular lncRNAs such as HOTAIR may have a comparable structural role. Polycomb Repressor Complex 2 (PRC2) is an evolutionarily conserved protein complex that has been estimated to associate with up to 20% of all known lncRNAs(36), and is heavily implicated in cancer pathogenesis(37). In particular, the recruitment of PRC2 by HOTAIR has been demonstrated to be a pivotal event in breast cancer development(32). Additionally, immunoprecipitation experiments have shown that breast cancer susceptibility gene 1 (BRCA1), which is essential for mediating the DNA damage response, interacts with a subunit of PRC2 to compete with HOTAIR for binding to the complex(38). Consistent with this finding, decreased expression of BRCA1 has been shown to result in augmented recruitment of PRC2 by HOTAIR in breast cancer cell lines(38). The significance of the antagonistic functional relationship between BRCA1 and HOTAIR in breast cancer remains unclear. Nevertheless, this parallel interaction of HOTAIR with PRC2 and BRCA1 highlights how a single lncRNA can serve as a central platform for multiple effectors to be brought together in space and time.

Genomic retargeting of PRC2

A distinct advantage of lncRNA-ribonucleoprotein complexes is that they can be targeted to specific genomic loci in order to activate or suppress transcriptional activities. Interestingly, HOTAIR is one of the first lncRNAs known to guide changes in gene expression in *trans* mode – i.e. at distant sites (as opposed to *cis*-acting transcripts, which act on neighbouring genes). The association of HOTAIR with PRC2 highlights one of the most exciting roles of lncRNAs as epigenetic regulators of gene expression. PRC2 is a chromatin modifying enzyme that promotes gene repression by methylating histone H3 on lysine 27 to promote gene repression(39), and its targeting illustrates how gene expression patterns can be altered in disease via modulation of genomic activity independent of any sequence alteration. Importantly, the overexpression of HOTAIR causes the reprogramming of PRC2

activity across the genome to specifically promote metastasis in breast cancer(32). In contrast, depletion of this transcript in tumour cells expressing a high basal level of PRC2 results in reduced invasiveness(40). These results indicate that the genome-wide retargeting of PRC2 induced by HOTAIR is somehow critical for metastatic progression.

Silencing of HOX-D

Amongst the target genes of PRC2 is that of homeobox (HOX)-D, a transcription factor that has been implicated in oncogenesis(41). The transcriptional silencing of HOX-D is likely to be a key step in the promotion of metastatic breast cancer by HOTAIR/PRC2(42). The indirect regulation of HOX-D activity by HOTAIR also has wider pathobiological implications. A fundamental role of lncRNAs at the molecular level is the regulation of gene transcription; previously, we have seen how HOTAIR can act as a molecular signal transducer in response to activation by c-Myc in gallbladder cancer(31). However, the functional interaction between HOTAIR and HOX-D in breast cancer illustrates how particular lncRNAs can also behave as 'top-level' regulators of key transcription factors involved in cancer signalling. Under normal physiological conditions, the use of PRC2 as an effector allows HOTAIR to exert its transcriptional regulation on a vast array of genes, including those in remote locations of the genome. However, this also means that widespread alterations in the genomic landscape can potentially result under pathological conditions.

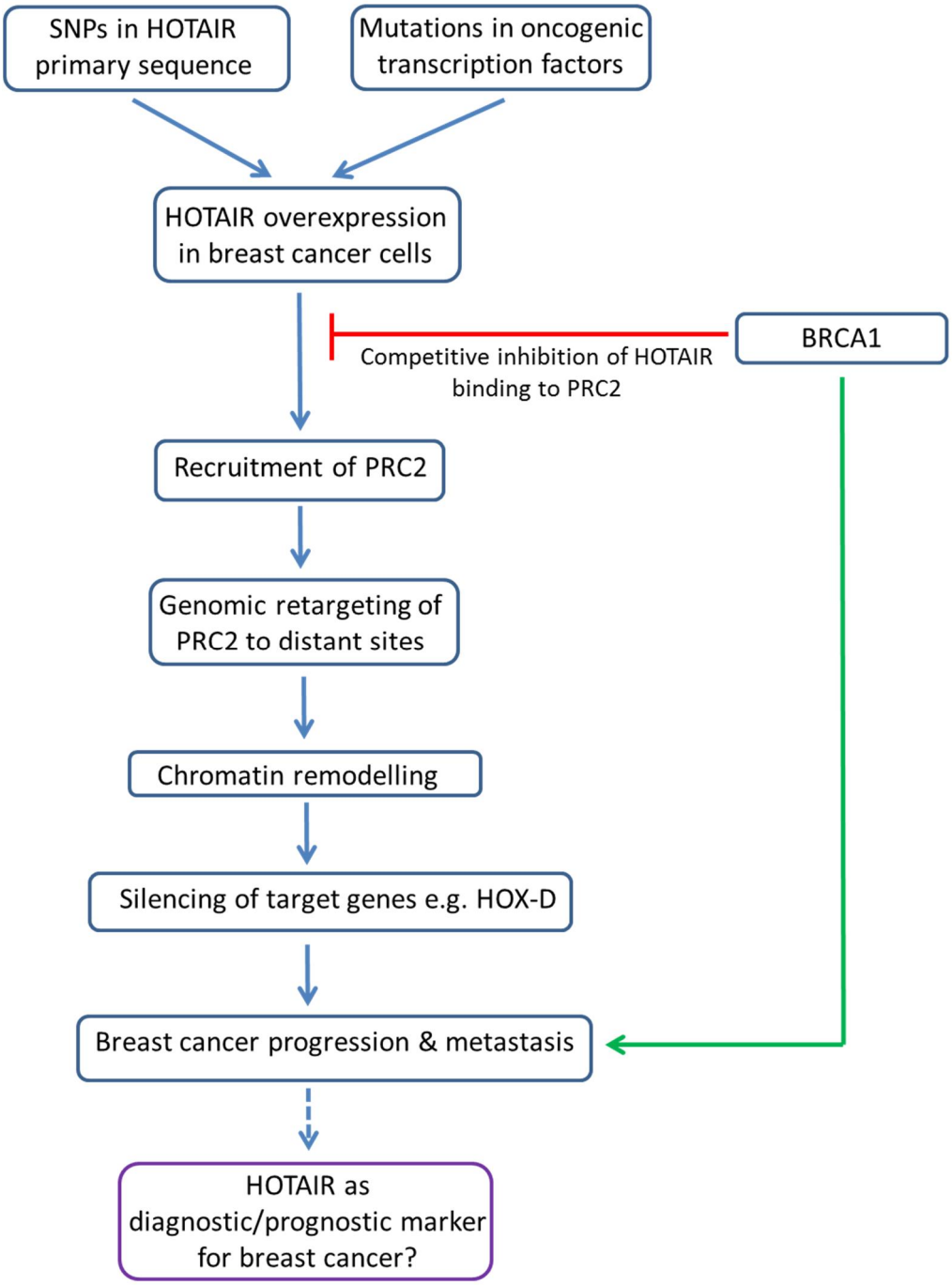

Figure 3. A flowchart illustrating some of the known mechanisms that the lncRNA 'HOTAIR' uses to induce breast cancer metastasis. HOTAIR expression is augmented in transformed cells as a result of genomic alterations such as SNPs in its primary sequence, or mutations in oncogenic transcription factors that it normally interacts with. HOTAIR can act as a scaffold and long-range guide for protein complexes; overexpressed HOTAIR causes increased genome-wide retargeting of polycomb complex (PRC2), which is involved in chromatin remodelling. This leads to silencing of multiple target genes, including the transcription factor HOX-D, highlighting the role of HOTAIR as a regulator of oncogenic transcription factors. These mechanisms ultimately promote breast cancer progression and metastasis, implying a potential role of HOTAIR as a diagnostic/prognostic indicator of breast cancer. The HOTAIR/PRC2 scaffold can also accommodate BRCA1, a gene critical for breast cancer pathogenesis. BRCA1 is a known competitive inhibitor of HOTAIR binding to PRC2, and can contribute to disease phenotype via independent mechanisms.

The impact of lncRNA dysfunction in cancer pathogenesis

Recent functional and mechanistic assessments of lncRNAs have revealed illuminating insights into their roles in cancer. However, what is the significance of lncRNA dysfunction in disease? After all, it must be remembered that lncRNAs are merely one of several different subclasses of ncRNA. For example, a large proportion of miRNAs has been found to be located in fragile regions of chromosomes associated with cancer(43), and aberrantly expressed in tumour cells(44). In particular, incidence of numerous cancers has been found to correlate with the overexpression of the miR-17-92 cluster(45). Likewise, miRNAs can participate in oncogenic and tumour suppressor pathways, contributing to disease phenotype through transcriptional and epigenetic mechanisms(46). In light of our limited understanding of the biogenesis of these non-coding RNA transcripts, and the somewhat arbitrary nature of our current classification system, it is likely that at least a small proportion of lncRNAs and miRNAs will have structural and functional uniformity. Moreover, given the extraordinarily large number of ncRNAs in humans and their involvement in virtually all signalling and developmental processes, it seems physiologically advantageous to ensure some functional redundancy within the mammalian ncRNA system.

However, it seems unlikely that lncRNAs are merely longer carbon copies of their counterparts. In fact, although only a small fraction of known lncRNAs has been functionally characterised, the evidence to date hints at the possibility that long and short ncRNAs contribute to cancer via distinct pathways to achieve the same desired outcome, in particular with relation to epigenetic regulation of gene expression. This can be illustrated by the use of mechanisms not classically associated with short ncRNAs, as exemplified by the aforementioned recruitment and targeting of chromatin modifying complexes such as PRC2, in addition to emerging mechanisms such as 'chromatin looping' by lncRNAs produced from enhancer regions within the genome(47). Additionally, a striking feature of lncRNAs is that while they typically display multiple modes of action, individual mechanisms employed by a single transcript could well be cancer type specific. To use just one example, crosstalk between different ncRNA classes is exemplified by some lncRNAs behaving as competitive

endogenous miRNA 'decoys' to inhibit miRNA activity. Ectopic expression of HOTAIR has been demonstrated to reduce miRNA-130a expression in gallbladder cancer tissues(31), and this particular pathobiological mechanism of HOTAIR is in direct contrast to its involvement in PRC2 retargeting observed in breast cancer. Furthermore, the multi-level crosstalk between lncRNAs, miRNAs, and transcription factors discussed earlier suggests that besides providing distinct pathways for inducing the onset and progression of cancer, different ncRNA subtypes can function synergistically in a highly integrated manner to promote disease. All of these observations argue against lncRNAs as functionally redundant replicas of short ncRNAs. But despite our awareness of such intricate signalling models, the sheer number of lncRNAs identified to date serves as a stark reminder that we are still a long way from understanding the full spectrum of their roles in pathology.

Future perspectives

Emerging epigenomic and bioinformatic approaches promise the identification of functional lncRNAs that remain hidden in the human genome(48). Furthermore, with the increasing ease of whole genome sequencing, as highlighted by the ongoing '100,000 Genomes Project', and the development of transgenic mouse models for single lncRNAs, significant headways are being made in characterising the pathobiological significance of lncRNAs in cancer. Importantly, there is increasing evidence to indicate a role for lncRNA dysfunction beyond cancer, such as in cardiovascular diseases(49) and neurological disorders(50). However, the relevant pathobiological mechanisms await experimental elucidation. Finally, understanding the precise roles of lncRNAs in pathology likely has important clinical applications. Considering the specific expression profiles of individual lncRNA transcripts in different tumour types, the notion of lncRNAs as diagnostic biomarkers and prognostic indicators of disease seems a very real possibility(51). Furthermore, one could envisage lncRNAs serving as targets for small molecule drugs in the not-too-distant future. Indeed, attempting to unmask the 'dark matter' of the genome in disease can at times resemble opening a Pandora's box, but amidst the many challenges and unanswered questions, the future is bright.

References

1. Crick F. Central dogma of molecular biology. Nature. 1970;227(5258):561-3.
2. Bartonicek N, Maag JL, Dinger ME. Long noncoding RNAs in cancer: mechanisms of action and technological advancements. Molecular cancer. 2016;15(1):43.
3. Meli M, Albert-Fournier B, Maurel MC. Recent findings in the modern RNA world. Int Microbiol. 2001;4(1):5-11.
4. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;75(5):843-54.
5. International Human Genome Sequencing C. Finishing the euchromatic sequence of the human genome. Nature. 2004;431(7011):931-45.
6. Irminger-Finger I, Kargul J, Laurent GJ. Non-coding RNAs: a novel level of genome complexity. Int J Biochem Cell Biol. 2014;54:286.
7. Consortium F, the RP, Clst, Forrest AR, Kawaji H, Rehli M, et al. A promoter-level mammalian expression atlas. Nature. 2014;507(7493):462-70.
8. Consortium EP, Birney E, Stamatoyannopoulos JA, Dutta A, Guigo R, Gingeras TR, et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature. 2007;447(7146):799-816.
9. Hangauer MJ, Vaughn IW, McManus MT. Pervasive transcription of the human genome produces thousands of previously unidentified long intergenic noncoding RNAs. PLoS Genet. 2013;9(6):e1003569.
10. Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y, et al. The landscape of long noncoding RNAs in the human transcriptome. Nat Genet. 2015;47(3):199-208.
11. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet. 2009;10(3):155-9.
12. Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, et al. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res. 2012;22(9):1775-89.
13. Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, et al. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev. 2011;25(18):1915-27.
14. Lyle R, Watanabe D, te Vruchte D, Lerchner W, Smrzka OW, Wutz A, et al. The imprinted antisense RNA at the Igf2r locus overlaps but does not imprint Mas1. Nat Genet. 2000;25(1):19-21.
15. Dinger ME, Pang KC, Mercer TR, Mattick JS. Differentiating protein-coding and noncoding RNA: challenges and ambiguities. PLoS Comput Biol. 2008;4(11):e1000176.
16. Almeida MI, Reis RM, Calin GA. MicroRNA history: discovery, recent applications, and next frontiers. Mutat Res. 2011;717(1-2):1-8.
17. Necsulea A, Soumillon M, Warnefors M, Liechti A, Daish T, Zeller U, et al. The evolution of lncRNA repertoires and expression patterns in tetrapods. Nature. 2014;505(7485):635-40.
18. Preker P, Nielsen J, Kammler S, Lykke-Andersen S, Christensen MS, Mapendano CK, et al. RNA exosome depletion reveals transcription upstream of active human promoters. Science. 2008;322(5909):1851-4.
19. Palazzo AF, Lee ES. Non-coding RNA: what is functional and what is junk? Front Genet. 2015;6:2.
20. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell. 2009;136(4):629-41.
21. Spizzo R, Almeida MI, Colombatti A, Calin GA. Long non-coding RNAs and cancer: a new frontier of translational research? Oncogene. 2012;31(43):4577-87.
22. Yan X, Hu Z, Feng Y, Hu X, Yuan J, Zhao SD, et al. Comprehensive Genomic Characterization of Long Non-coding RNAs across Human Cancers. Cancer Cell. 2015;28(4):529-40.
23. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A. 2002;99(24):15524-9.
24. Mercer TR, Dinger ME, Sunkin SM, Mehler MF, Mattick JS. Specific expression of long noncoding RNAs in the mouse brain. Proc Natl Acad Sci U S A. 2008;105(2):716-21.
25. Clemson CM, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, Chess A, et al. An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. Mol Cell. 2009;33(6):717-26.
26. Hajjari M, Salavaty A. HOTAIR: an oncogenic long non-coding RNA in different cancers. Cancer Biol Med. 2015;12(1):1-9.

27. Clark MB, Mercer TR, Bussotti G, Leonardi T, Haynes KR, Crawford J, et al. Quantitative gene profiling of long noncoding RNAs with targeted RNA sequencing. Nat Methods. 2015;12(4):339-42.
28. Lim DH, Maher ER. Genomic imprinting syndromes and cancer. Adv Genet. 2010;70:145-75.
29. Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, et al. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc Natl Acad Sci U S A. 2009;106(23):9362-7.
30. Diskin SJ, Capasso M, Schnepp RW, Cole KA, Attiyeh EF, Hou C, et al. Common variation at 6q16 within HACE1 and LIN28B influences susceptibility to neuroblastoma. Nat Genet. 2012;44(10):1126-30.
31. Ma MZ, Li CX, Zhang Y, Weng MZ, Zhang MD, Qin YY, et al. Long non-coding RNA HOTAIR, a c-Myc activated driver of malignancy, negatively regulates miRNA-130a in gallbladder cancer. Molecular cancer. 2014;13:156.
32. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature. 2010;464(7291):1071-6.
33. Lu KH, Li W, Liu XH, Sun M, Zhang ML, Wu WQ, et al. Long non-coding RNA MEG3 inhibits NSCLC cells proliferation and induces apoptosis by affecting p53 expression. BMC Cancer. 2013;13:461.
34. Shen XH, Qi P, Du X. Long non-coding RNAs in cancer invasion and metastasis. Mod Pathol. 2015;28(1):4-13.
35. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Mol Cell. 2011;43(6):904-14.
36. Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, et al. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(28):11667-72.
37. Chisholm KM, Wan Y, Li R, Montgomery KD, Chang HY, West RB. Detection of long non-coding RNA in archival tissue: correlation with polycomb protein expression in primary and metastatic breast carcinoma. PLoS One. 2012;7(10):e47998.
38. Wang L, Zeng X, Chen S, Ding L, Zhong J, Zhao JC, et al. BRCA1 is a negative modulator of the PRC2 complex. EMBO J. 2013;32(11):1584-97.
39. Sparmann A, van Lohuizen M. Polycomb silencers control cell fate, development and cancer. Nat Rev Cancer. 2006;6(11):846-56.
40. Wan Y, Chang HY. HOTAIR: Flight of noncoding RNAs in cancer metastasis. Cell Cycle. 2010;9(17):3391-2.
41. Shah N, Sukumar S. The Hox genes and their roles in oncogenesis. Nat Rev Cancer. 2010;10(5):361-71.
42. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, et al. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell. 2007;129(7):1311-23.
43. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A. 2004;101(9):2999-3004.
44. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature. 2005;435(7043):834-8.
45. Mendell JT. miRiad roles for the miR-17-92 cluster in development and disease. Cell. 2008;133(2):217-22.
46. Garzon R, Calin GA, Croce CM. MicroRNAs in Cancer. Annu Rev Med. 2009;60:167-79.
47. Melo CA, Drost J, Wijchers PJ, van de Werken H, de Wit E, Oude Vrielink JA, et al. eRNAs are required for p53-dependent enhancer activity and gene transcription. Mol Cell. 2013;49(3):524-35.
48. Chen X, Yan CC, Zhang X, You ZH. Long non-coding RNAs and complex diseases: from experimental results to computational models. Brief Bioinform. 2016.
49. Archer K, Broskova Z, Bayoumi AS, Teoh JP, Davila A, Tang Y, et al. Long Non-Coding RNAs as Master Regulators in Cardiovascular Diseases. Int J Mol Sci. 2015;16(10):23651-67.
50. Salta E, De Strooper B. Non-coding RNAs with essential roles in neurodegenerative disorders. Lancet Neurol. 2012;11(2):189-200.
51. Qi P, Du X. The long non-coding RNAs, a new cancer diagnostic and therapeutic gold mine. Mod Pathol. 2013;26(2):155-65.