



# Long non-coding RNAs: new opportunities and old challenges in cancer therapy

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The investigation of long non-protein-coding RNAs (lncRNAs) has become a key area in biological and biomedical research. This is the result of sequencing and analysis of the human genome, which showed that only around 2% encodes proteins, together with the analysis of RNA transcripts (the transcriptome), which showed that a further 80% or so of the genome is actively transcribed (1,2). Consequently, it is now clear that most of the human genome largely encodes non-protein-coding RNAs, most of which, at greater than 200 nucleotides, are classified as lncRNAs. Although we are only just beginning to analyse this vast number of transcripts, it is already evident that many lncRNAs play crucial roles in human molecular cell biology ranging from providing essential frameworks for RNA processing, through the epigenetic control of gene expression, to blocking and regulating cell signalling pathways [reviewed by Morris and Mattick (3)]. It is not surprising therefore that dysfunction in many lncRNAs has already been strongly implicated in oncogenesis and in the development of resistance to cancer therapy (4). Such a profound advance in our understanding of human cell physiology and pathology will clearly present many opportunities for the development of entirely novel cancer therapies.

There are already multiple studies that indicate that many lncRNAs can act as tumour suppressors, and many others as oncogenes (4). As for protein tumour suppressors and oncogenes, this provides new opportunities for specific cancer therapies through restoring and enhancing lncRNA

tumour suppressor activity where that has been lost, and through inhibiting lncRNA oncogenic activity where that contributes to oncogenesis and/or resistance to therapy. The GAS5 lncRNA, for example, has been shown to regulate proliferation and survival in lymphoid cells (5), shows tumour suppressor activity in a wide range of cancers (6-9) and can restore sensitivity to chemotherapeutic agents (6). Although the tumour suppressor activity of GAS5 appears to be mediated by several different interactions (6), as for many other lncRNAs, this activity in breast and prostate cancer cells is predominantly due to interaction of a stem-loop RNA sequence within GAS5 exon 12 with glucocorticoid/related hormone receptors (10). This part of the GAS5 lncRNA acts as a hormone response element mimic (HREM) and blocks the endocrine survival signal(s) otherwise mediated through these pathways. Thus this part of the GAS5 lncRNA acts as a decoy and prevents the hormone/hormone receptor complex from binding to its usual target genes (10,11). If this tumour suppressor activity can be restored with a short nucleotide sequence mimicking the GAS5 HREM sequence, this would be a much more realistic approach than re-introducing the whole of GAS5 by gene therapy, and recent work has demonstrated proof of principle of this oligonucleotide approach (12). The introduction of a 23-base oligomer with the HREM sequence into a range of breast cancer cell lines (including triple-negative cell lines) induced apoptosis and reduced clonogenicity in a manner that closely replicated the effects of GAS5 lncRNA itself, including the sensitivity

of these effects to a specific loss-of-function mutation (12). These results clearly encourage analysis of the effects of oligonucleotides designed to mimic critical sections of other tumour-suppressive lncRNAs.

Encouraging initial observations have also been made in logically complementary strategies intended to inhibit oncogenic lncRNAs. Downregulation of lncRNAs, including those localised in the nucleus, can be efficiently accomplished using antisense oligonucleotides (ASOs) (13). metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), for example, is a relatively abundant lncRNA that is upregulated in several cancers [reviewed by Ren *et al.* (14)]. Downregulation of MALAT1 has been achieved *in vivo* in a mouse model of breast cancer using ASOs, resulting in altered gene expression and RNA splicing together with a substantial reduction in metastasis (15).

These studies and many others underline the potential of oligonucleotide-based therapy to produce highly specific modulation of the expression of lncRNAs with clear applications in cancer therapy, and, naturally, this prospect has driven improvements in the technology required to realise this potential in real clinical applications. Although other oligonucleotide-based therapies have already produced significant successes in pre-clinical studies, clinical trials and some fully approved clinical applications [e.g., (16)], substantial challenges remain. Most of these challenges are familiar from other types of cancer therapy: How can maximal penetration of cancer cells be achieved? How can tumours be specifically targeted with minimal effects on healthy tissues? How can stability be improved for oligonucleotides exposed to host degradative enzymes? Given the considerable incentive of molecule-specific lncRNA cancer therapy, it is likely that these problems will be overcome. Significant improvements in oligonucleotide stability and cell penetration have been made through changes in nucleotide chemistry (17), and by attachment to peptides activated by the acidic tumour environment (18), for example. Advances in nanoparticle technology and immune-targeting of tumour antigens have, in turn, improved the specificity of oligonucleotide delivery (19).

Although oligonucleotide therapy offers exquisite specificity in targeting lncRNAs, the development of lncRNA-targeting small molecules is also realistic. Fatemi and colleagues (20) screened a library of phytochemical compounds for molecules interfering with the interaction of lncRNA BDNF-AS (BDNF-antisense RNA) with EZH2 (enhancer of zeste homolog 2—a key enzyme controlling

epigenetic modification of chromatin) and successfully identified ellipticine, which up-regulated BDNF transcription. Similar antisense lncRNAs are frequently involved in the epigenetic control of gene expression, and targeting of this class of lncRNAs offers particular promise for the development of highly specific cancer therapies.

Although the lncRNA field is still in its early stages—after all, over 50% of the genome has yet to be analysed in detail for its function—it is clear that this area presents almost unprecedented opportunities for novel cancer therapies. The appreciation of this potential is rapidly growing and should result in much-needed improvements for patients before long.

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