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Supplementary information

Long noncoding RNAs in cancer metastasis

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Table 1. Methodological considerations when studying lncRNAs

Method	Effect on RNA transcript	Effect on DNA locus	Additional Considerations
RNA interference	Transcript depletion through RISC	Generally not impacted in mammalian cells	Potential for widespread off target effects[1]
Genetic locus deletion	Ablates transcript	Removes potential *cis* regulatory sequences	Function may be ascribed to either RNA transcript or DNA sequence[2]
Promoter deletion	Disables transcription	Removes local regulatory sequences	Promoter sequences may be used as enhancer or silencer of distant genes[3,4]
Insertion of transcription termination signal	Prevents production of mature transcript	Minor genetic modification of DNA sequence	The process of transcription is retained, depending on location of poly-adenylation signal insertion[5]
CRISPR interference	Inhibits transcriptional initiation and elongation using engineered dCas9-repressor fusions	Deposits H3K9me3 heterochromatin at promoter region	Narrow window of activity allows perturbation of complex lncRNA loci[6]. Can epigenetically silence enhancers[7].
Splice site mutagenesis	Disrupts splicing of multi-exon transcripts	Potential deletion of key *cis* regulatory elements	Allows transcription of lncRNA locus. LncRNA function might not depend on mature primary transcript[8]
Antisense oligonucleotides	Primarily depletes nascent RNA through RNAse-H and XRN2	Not directly impacted	Off target effects and *in vivo* toxicity require careful design of ASO agents[9,10]
CRISPR-Cas13 direct RNA targeting	Guide RNA-directed RNA cleavage	Not directly impacted	Greater specificity than RNAi[11], but emerging method for lncRNAs with variable knockdown efficiency [12]
Transgenic overexpression	Overexpression typically at supraphysiologic levels, unless local regulatory elements are retained[13]	Genetic integration may activate local gene expression.	Approach assumes *trans* mechanism of action[14]
CRISPR activation	Promotes transcription using engineered dCas9-transactivator fusions	May generate euchromatin modifications	Can potentially activate enhancer regions in addition to gene transcription [15]
CRISPR display ectopic localization	Enables guide RNA-directed localization of exogenous lncRNA transcript fusions	Not directly impacted	Assumes *trans* mechanism of action and efficacy may be limited by lncRNA structure and size[16]

Overview of commonly used and emerging methods for loss of function or gain of function experimentation to study lncRNAs. Each method entails particular ramifications for RNA and DNA locus disruption that should be carefully considered when interpreting results from these assays. Additional discussion can be found in prior reviews[17,18].

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