From the Dark Transcriptome: Engineered lncRNAs as Controllers of Inflammation

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Background: Long non-coding RNAs (lncRNAs) comprise over one-third of the human transcriptome and represents a major component of the ‘dark transcriptome’. This extensive repertoire of non-protein-coding RNAs perform vital regulatory functions in cells without translation into protein. Conventionally used as diagnostic markers or small molecule drug targets, lncRNAs have never been exploited for exogenous therapeutic drug usage. Method: To demonstrate their utility, we mined the human transcriptome to develop three macrophage-related lncRNA as therapeutic drugs that treat systemic inflammation. We re-engineered three lncRNA from the transcriptome—GAPLINC, MIST and DRAIR—and successfully deployed them to treat acute inflammation in lipopolysaccharide-challenged animals. For each lncRNA, we established a new in vitro transcription synthesis and high-performance liquid chromatography purification workflow. Then, we optimized lncRNA isoform, 5’-cap, 3’ poly(A) tail and chemical base modifications to improve specificity and performance. In addition to sequence and isoforms, we demonstrate that performance and specificity are highly dependent on the type of modification. Further, we optimized lncRNA in vivo delivery using a lipid nanoparticle system. Results: Using this pipeline and delivery platform, we demonstrated that each lncRNA reduced lipopolysacchrideinduced inflammation uniquely by specifically regulating distinct subset of cytokines in cultured mouse macrophages and mice. Notably, chemically modified lncRNAs improved potency by up to two times compared to the unmodified version of the lncRNAs. While GAPLINC and DRAIR decreased the transcription of IL-1β and IL-6, respectively, MIST attenuated TNFα production posttranscriptionally. Additionally, we showed that reengineered GAPLINC reduced LPS-induced inflammation in human monocytes, suggesting the clinical potential of this approach. Conclusion: These findings showcase the utility of lncRNAs for treating acute inflammation and open opportunities for the development of lncRNA-based therapies to treat other conditions.