RNA as a small molecule drug target: Discovery of selective RNA-binding small molecules by affinity-selection mass spectrometry

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Abstract

Approximately 60% of DNA is transcribed as RNA, but only 1-2% codes for proteins. There is evidence that the remaining non-coding RNA (ncRNA) is involved in the regulation or execution of cellular processes, and has been genetically linked to human diseases and traits in similar numbers as the protein-coding genes. Understanding the function of ncRNA and its interactions with small molecules will likely open up new therapeutic approaches for various diseases.

To study ncRNA as a small molecule drug target, we first investigated the use of the Automated Ligand Identification System (ALIS). ALIS is an affinity-selection MS platform capable of high-throughput screening for small-molecule binding, and has been routinely used to detect thousands of protein-small molecule interactions. We successfully adapted ALIS for detection of RNA-small molecule interactions with regulatory ncRNA bacterial riboswitches and known small molecule drug leads, and used ALIS to characterize these RNA-ligand binding events.

We next identified over 40 ncRNA ligands from a range of classes and disease areas. These ncRNA targets were screened in ALIS against chemically diverse small molecule libraries, functionally annotated from phenotypic screens, and libraries enriched in RNA-binding properties (60,000+ compounds total). We have generated millions of screening data points from which we have identified new drug-like small molecules that bind to ncRNA. By further studying the structural conformation and functional consequences, we are revealing new mechanisms involving small molecule-ncRNA interaction. Here, we outline our results and discuss their implications for small-molecule drug discovery efforts.

Introduction

Why non-coding RNA?

Non-coding RNAs (ncRNA) are involved in regulation of transcription, translation, RNA modifications, and alteration of mRNA stability. These roles of ncRNA suggest that targeting RNA is a potential avenue for discovery of novel therapeutics in all areas of human biology, including cancer, metabolic disease, and many other disorders involving gene regulation and epigenetics.

Methods

ALIS: Automated Ligand Identification System

• Fully automated, online, label-free affinity selection technology based on two-dimensional SEC-RP/SEC separations coupled to high resolution / accurate mass (HR/AM) mass spectrometry
• ALIS first separates protein-ligand complexes by SEC, then denotes the complexes and detects previously bound ligands by LC-HR MS
• Output of ALIS screening mode is binary Yes/No binding

Proof-of-Concept: Riboswitches

The ALIS platform can detect selective binding of native ligands to noncoding regulatory RNA elements (riboswitches)

Results: HTS for RNA binders

42 RNA targets from various classes screened against a set of 50,000 diverse compounds

Results: Characterizing an RNA binder

Functional and structural studies of a novel binder to the FMN riboswitch

Conclusions

• The RNA-focused Library was successful in yielding a higher number of binding compounds
• Cheminformatics identifies structural chemotypes favorable for RNA-binding. These can be used to predict RNA targets during drug development programs.
• 30 of 42 RNA targets have at least 1 selectively binding compound. Functional studies for these selective binders is currently on-going.
• Structural and functional studies reveal structurally distinct small molecules can bind an RNA target and result in different RNA conformations, and therefore different phenotypes. These findings suggest the diversity of small molecule-RNA interactions and the importance of structural and functional follow-up.