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Therapeutic Targeting of Structural RNA Motifs in Viral RNA Genomes

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Introduction

Viral RNA genomes have evolved functional motifs, which act at different stages of their life cycle. These unique structural domains, their interactions and association with host proteins and ligands, together orchestrate the multifunctionality of viral RNA genomes. Often, long-range RNA-RNA interactions bring regulatory elements into proximity, changing our view of a functional viral RNA genome from a linear molecule to one whose three-dimensional structure is an important contributor during their life cycle [1,2]. Because of their key and versatile roles in viral molecular processes, such as protein synthesis, splicing, transcriptional regulation and replication, viral genomes and virus-coded RNAs, i.e. subgenomic RNAs, pregenomic RNAs, can now be considered attractive therapeutic targets. Thus, understanding the 3D structure of viral RNAs, how their conformation correlates with disease progression, and whether this is therapeutically accessible, is a clear priority.

RNA as a Therapeutic Target

RNA structure resembles that of DNA as it is composed of nitrogenous bases linked to hydrophobic sugar structures, but at the same time RNA also mimics proteins in that it folds into intricate hydrophilic pockets suitable for binding small molecule ligands. These attributes give RNA very attractive characteristics for use in therapeutic intervention. Yet, RNA remains an under-exploited therapeutic target when compared to other biologically important macromolecules, mainly due to its structurally dynamic and chemically unstable nature.

A crucial aspect of targeting viral RNAs with small molecules is identification of functional targets within the large multidomain viral genome. Extensive work in the area of defining optimal sites for RNA targeting has suggested that regions where there is deviation of the A-form helix are ideal for small molecule binding. Such deviations create various classes of secondary structures, such as hairpin loops, internal loops, and bulged regions [3]. Thus, an absolute prerequisite for aiming at RNA is an accurate prediction of the pattern of base pairing, or secondary structure. Chemoenzymatic probing analysis, i.e. selective 2'hydroxyl acylation analyzed by primer extension (SHAPE) [4], conventional chemical mapping using DMS, DEPC, kethoxal [5] and enzymatic methods [6] are sufficient in most cases to select the correct regional fold, but for some RNA sections, additional information can be required. A component for any viral RNA inhibitor discovery platform is also the confirmation that target RNA motifs remain biologically functional in isolation. If structural elements are formed via close-range base pairing in the context of the entire RNA, these should also base pair in the context of smaller fragments.

Important limitations are the difficulty encountered in predicting long-range and tertiary structure interactions that contribute to the intricate folding of RNA. Although those more complex aspects of RNA structure are computationally difficult to predict, some algorithms have been developed that allow for pseudoknots or are able to predict tertiary structure [7]. Pseudoknots and kissing hairpins have been observed in a number of functional RNA sequences, and the genomes of viral RNAs, in which they have been shown to be involved in unique mechanisms of viral translation initiation, elongation and frameshifting [8-10].Thus, ignoring these tertiary interactions would result in inaccurate structure predictions. Additionally, these composite RNA domains and putative long-range RNA-RNA interactions can be verified by application of bifunctional structural probe that combine a recognition and effector motifs [11,12]. Site-directed through-space cleavage can be accomplished by linking an Fe(II)–EDTA moiety to a molecule that binds selectively to a user-defined site in an RNA. This approach can yield a large number of high-quality, medium-distance constraints that together with experimentally established RNA secondary structure can be incorporated into modeling programs to build atomic-resolution 3D models of the studied RNA [13]. As a confirmative strategy, antisense-interfered SHAPE (aiSHAPE) can be applied to verify the RNA tertiary interactions. In this method, displacing one strand of RNA duplex, i.e. in pseudoknot, by hybridizing an antisense oligonucleotide would disrupt long-distance interactions and be characterized by enhanced electrophilic sensitivity of the displaced nucleotides [1].

Combining all these methodologies yields high-resolution information regarding the structure of RNA. Gaining the in-depth insight into the RNA molecular crevices allows it to be more precisely targeted with small molecules aiding future development of novel antiviral therapeutics.

Small Molecule Microarrays as a Novel Strategy for RNA Targeting

Successful strategies to identify small molecules targeting RNA have markedly lingered behind equivalent methods for molecules

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that interact with proteins. Also, robust technologies that screen large, unbiased chemical libraries against structured RNA motifs are relatively rare. Although chemoenzymatic probing and conventional probing techniques have been successfully applied to resolve the conformation of viral RNAs, there are limited publications indicating their use in guiding identification of small molecules targeting cis-acting regulatory motifs. To this end, only few approaches that identify RNA-binding small molecules have been described. Herman et al. [14] targeted the internal ribosome entry site (IRES) of the hepatitis C virus (HCV). Butcher et al. [15] and Al-Hashimi et al. [16] targeted the franeshift and transactivation response (TAR) elements from HIV-1, respectively.

We have recently employed a strategy to screen a large unbiased library of drug-like small molecules in a small molecule microarray format (SMM) against an RNA target [17]. This strategy identified a novel chemotype that selectively targets the HIV transactivation response (TAR) RNA hairpin in a manner not dependent on cationic charge. The candidate thienopyridine binds to and stabilizes the TAR hairpin with a Kd of 2.4 uM. Structure activity relationships demonstrate that this compound achieves activity through hydrophobic substituents on a heterocyclic core, rather than cationic groups typically required. Selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) analysis was performed on a 365-nucleotide sequence derived from the 5' UTR of the HIV-1 genome to determine global structural changes in the presence of the molecule. The interaction of this compound could be mapped to the TAR hairpin without broadly disrupting any other structured elements of the 5' UTR. Cell-based assays indicated that this novel small molecule inhibits HIV-induced cytopathicity in the micromolar range, while cytotoxicity was not observed at concentrations of 1 mM. This unbiased approach can easily be applied to other viral RNAs. The ease with which SMM assays are prepared enables screening multiple RNA structural motifs in a relatively short timeframe, with minimal assay optimization and small quantities of target RNA. Because the small molecule is linked to the microarray surface via an amine or alcohol functionality, "tagging" identified molecules (e.g. with a fluorescent dye, NMR spin label, or chemically reactive moiety for footprinting studies) at this functional group is unlikely to disrupt target RNA binding. Thus, in addition inhibiting RNA function, such bifunctional molecules can be exploited to probe complex RNA structures.

Targeting RNA-Protein Interactions

As the three-dimensional conformation of RNA supports formation of intricate secondary and tertiary interactions, RNA motifs often mediate highly specific sequestration of various effector molecules, i.e. viral and/or host protein factors. Binding of small molecule ligands to RNA motifs might influence its functionality not only by inhibiting RNA catalysis or forcing an alternative folding on the RNA, but also by preventing binding of the biologically important macromolecule. Accordingly, to successfully design antiviral therapeutics, it is of utmost importance to define the mechanism of RNA-protein interactions that can be also targeted with small molecules. Various methodologies have been developed to identify the position of protein binding on RNA, including ultraviolet (UV) crosslinking and immunoprecipitation (CLIP) [18], photoactivable ribonucleoside enhanced CLIP (PAR-CLIP) [19], individual nucleotide resolution CLIP (iCLIP) [20]. The experimentally defined physical maps of RNA-protein interactions can be subsequently integrated with RNA structural information for more precise small molecule targeting.

Recently, Bell et al. [21] reported the application of a target-based fluorogenic destabilization assay to identify small molecules, which modulate the interaction between Gag and packaging domain (PSI) elements in HIV-1. They demonstrated that one of the identified small molecule ligands exhibited potent inhibitory activity in a viral replication assay by binding to the tetraloop of stem-loop 3 (SL3) in PSI. The authors employed circular dichroism (CD), fluorescence melting and 1H NMR spectroscopy to provide structural insight on the binding of the compound to the RNA SL3 of the HIV-1 packaging domain.

Results discussed here represent a proof of concept for the identification of specific small molecule inhibitors of RNA and RNA-protein interactions that are critical for viral life cycle. Since viral RNA targets offer great potential as a unique approach to antiviral intervention, these approaches open a novel paradigm for drug development.

Conclusions

Advances in the use of biological drugs have considerably enhanced the scope of therapeutic targets for a variety of human diseases. In the RNA world, secondary structure and long-range tertiary interactions are crucial mediators of interactions with regulatory proteins or other nucleic acids. Since RNA tertiary structure also mediates the activity and function of viral genomes and virus-coded RNAs, the cis- and trans-acting RNA structural motif are receiving increased attention as a vast source of targets that might be antagonized by small molecule-derived therapeutics. Thus, understanding the 3D structure of viral RNAs, how their dysregulation is correlated with disease progression and whether this is amenable to therapeutic intervention, is of utmost importance for clinical advances.

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