Discovery of mRNA G-quadruplex binders through high-throughput Affinity Selection Mass Spectrometry screening and comprehensive hit profiling

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Overview

- Case study on the applicability of assay systems for RNA-targeted drug discovery.
- Performed RNA binder screening and hit profiling.
- Successfully identified novel small-molecule binders targeting mRNA G-quadruplex (G4) with cellular activity.

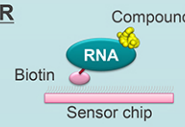
Introduction

RNA-targeted small molecule drug discovery is a promising and challenging field. High-throughput screening (HTS) is a powerful tool for identifying RNA-targeted drugs, but it presents many challenges, including the validation of the RNA sequences used for screening, the lack of a standard screening cascade, and hit validation strategies. To test the feasibility of multiple assay systems for RNA-targeted drug discovery and to examine the correlation between individual assays, we performed an RNA binder screening and hit validation targeting the G4 structure. Our study demonstrated for the first time that HTS by ASMS competition binding assay and compound profiling using RNA recognition antibody is useful for RNA-targeted drug discovery. It was also suggested that having a platform of multiple biophysical methods is the key to success in advancing RNA drug discovery.

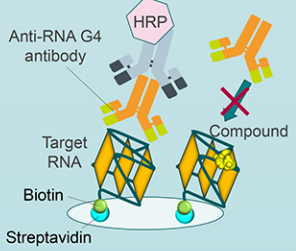
Methods

ASMS binding assay

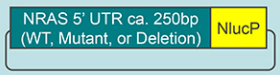
SPR



Anti-RNA G4 antibody affinity assay



Cell-based reporter assay



a

Name	Sequence
NRAS WT	UGU**GGGAGGGGC**GGGUCU**GGG**
NRAS Mutant	UGU**GUGAGUGUC**GUGUCU**GUG**
NRAS Antisense	CCCAGACCCGCCCCUCCCACA
NRAS WT modified	UGU**GGGA**UGGGGC**GGG**UCU**GGGA**

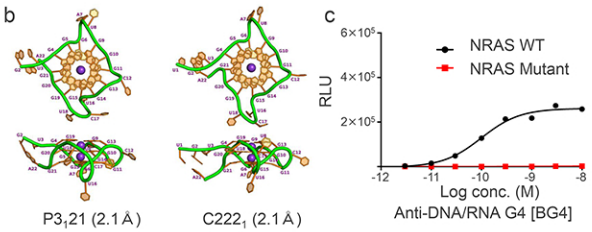


Figure 1. Confirmation of NRAS mRNA G4 structure. Information of NRAS mRNA sequences (a). Crystal structure analysis using the NRAS WT modified sequence confirmed that the structure of NRAS mRNA G4 was equivalent to the reported structure (b). Anti-DNA/RNA G4 antibody (BG4) selectively recognized the NRAS WT compared to the NRAS mutant (c).

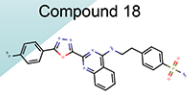
a

Primary screening
- ASMS competition binding assay (NRAS WT and Compound 18)
- 25K compounds (mixture of 10 compounds per well), each 1 or 3 μM

Deconvolution and Retest
- ASMS competition binding assay
- 78 compounds, 3 and 10 μM

Profiling
- ASMS direct binding assay
- SPR binding assay
- anti-RNA G4 antibody affinity assay
- Cell-based reporter assay
- 55 compounds classified in 36 clusters were tested in all assays

Compound 18



Balaratnam et al., Cell Chem Biol. 2023 Jun 15;30(6):643-657.

Screening Hit
- ✓ 36 of the 55 compounds were successfully yielded Kd values in ASMS direct binding assay
- ✓ 18 of the 55 compounds exhibited Kd or IC_{50} values in all ASMS-based direct binding assay, SPR binding assay, and anti-RNA G4 antibody affinity assay
- ✓ 2 of the 55 compounds showed the activity in cell-based reporter assay

b

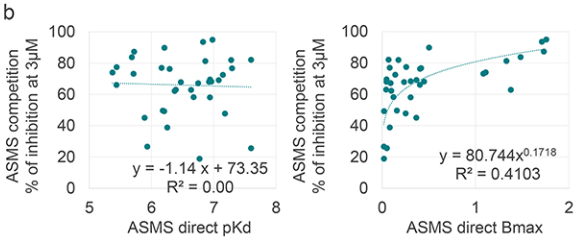


Figure 2. Overview of HTS. HTS cascade is shown in (a). 36 of the 55 compounds were successfully yielded measurable Kd values in the ASMS direct binding assay. The correlation between ASMS competition binding and direct binding was analyzed. There was no clear correlation between % of inhibition at 3 μM and pKd, but there was a weak correlation between % of inhibition at 3 μM and Bmax (b).

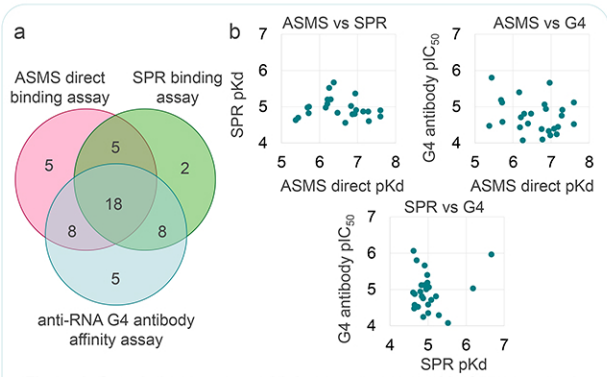


Figure 3. Correlation among multiple assay systems. 39 of 55 compounds showed binding activity in multiple assay systems. On the other hand, 12 of 55 compounds showed activity only in certain assays (a). The correlation between the activity in each assay was analyzed, but there was no clear correlation. In the SPR assay, non-specific binding at high compound concentrations prevented accurate measurement of Kd values (b).

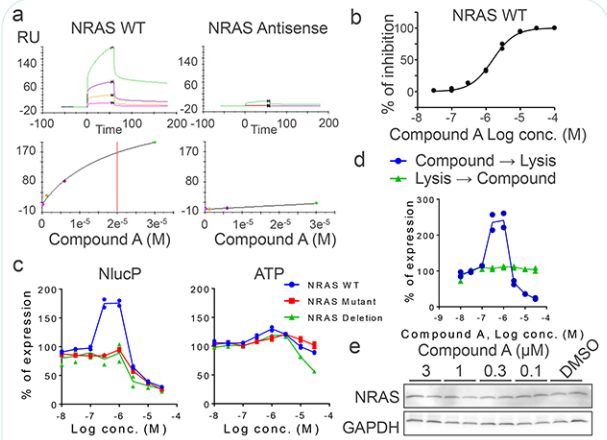


Figure 4. Profiling of Compound A Compound A showed clear binding activity in SPR (a) and anti-RNA G4 antibody affinity (b) assays in addition to ASMS direct binding assay. In cell-based reporter assay, there was a marked enhancement of the NlucP signal upon treatment with 0.3 μM and 1 μM for 6 h (c). When Compound A was added after cell lysis, there was no effect on the signal suggesting that the compound does not act directly on the NlucP luciferase activity (d). Endogenous NRAS protein expression levels in MCF7 were not altered by treatment with Compound A (e).

Conclusion

Our study successfully identified small-molecule binders targeting NRAS mRNA G4. Although we did not find a clear correlation between multiple binding assays, combining them allowed us to select appropriate Hit compounds. Selectivity tests among different types of RNA G4 and structure-activity relationship studies are under consideration as the next steps. RNA G4 focused small-molecule library is also expected to be developed.

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