Identification and characterization of small molecules inhibiting RNA targets: miRNA

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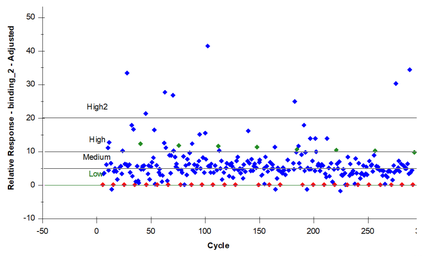
Abstract

RNA plays a key role in transcription[1] and translation:[2] gene regulation, proliferation, apoptosis, and development. The biological function is linked to the unique secondary and tertiary structures.[3]
Many diseases are associated with dysregulation of RNA functions,[4,5] so there is a growing interest in RNAs as drug targets for novel therapies or targeting undruggable proteins. The most used modalities are antisense oligonucleotides (ASOs), CRISPR gene editing, and more recently small molecules.[6]
Although ASOs and CRISPR are highly specific, they bring some significant challenges such as delivery and adverse reactions. Small molecules are attractive because drug-like properties can be optimized by classical medicinal chemistry.[7]
Targeting RNAs with small molecules is a promising modality for oncology, cardiovascular diseases and CNS disorders. In particular, dys-regulation of microRNAs play key roles in the genesis of the different diseases and thus have been targeted by small molecule inhibitors recently. Here, we describe the setup of a flowchart to identify and optimize new efficient inhibitors of microRNA. Starting with SPR to select and characterize a group of small molecules that bind specifically to pre-miR with high affinities, we validated their inhibitory activity on miR maturation and function with biochemical and cellular assays. The approach demonstrates a miRNA targeting platform that could be applied to other oncogenic miRNA targets.

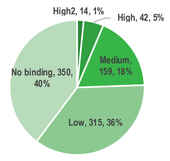
Results

HTS screening with SPR: Binding Level Screen

- Annealing
- Biot-pre-miRNAs immobilization
- Ref compound for set up
- 3 pre-miRNAs tested in parallel
- Batches of 800 -1000 cmpds @ 1 or 2 conc



Category	Response level (R)
High2; Multibinder	R > 100% Rmax
High	50% Rmax < R < 100% Rmax
Medium	25% Rmax < R < 50% Rmax
Low	R < 25% Rmax
No binding	R ≤ 0



✓ Classification of the binders

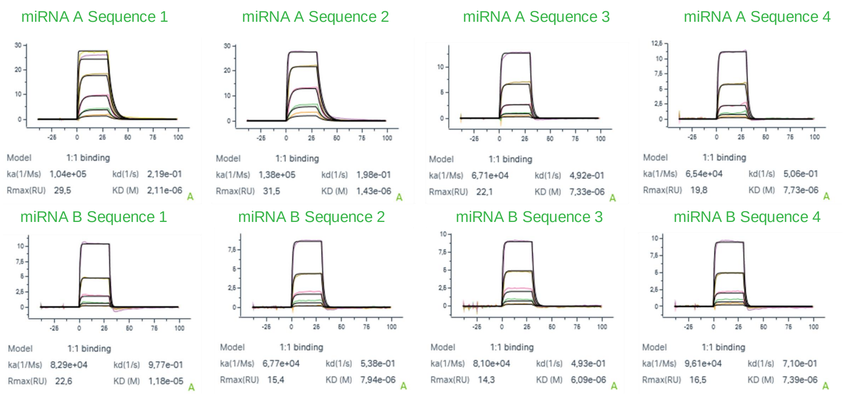
Hits characterization and profiling : Multi-Cycle Kinetic

Kinetic fit for Compound A

Fc2*

Ch1	miRNA A Seq 1	Ch5	miRNA B Seq 1
Ch2	miRNA A Seq 2	Ch6	miRNA B Seq 2
Ch3	miRNA A Seq 3	Ch7	miRNA B Seq 3
Ch4	miRNA A Seq 4	Ch8	miRNA B Seq 4

- Affinity & Kinetic determination
- Hits ranking K_D
- Hits profiling: selectivity vs other miRNAs



High-throughput SPR: simultaneous analysis of multiple targets and compounds

	One channel-based SPR	High-throughput SPR
Time (50 compounds run in dose-response)	**70 hrs**	**15 hrs**
Compounds per assay	Max 384/ 1 cmpd per cycle	32Pro = **12 x 384** 8K = **4 x 384** 8 cmpds per cycle in parallel
Number of tagets analysed in parallel	3	32Pro: up to 24 8K: up to 8

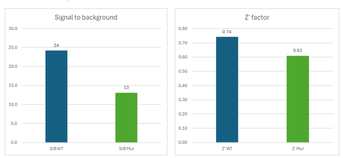
Specificity assessment by SPR

- Counter screen performed against Ribonuclease of interest
- Kinetic analysis reveals subtle differencies in binding between DICER and miRNA providing further insights into MoA

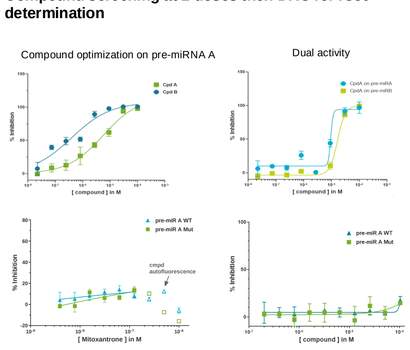
1 - Fluorescent assay to measure DICER activity → 2 - Validation of the miRNA cleavage by Bioanalyzer → 3 - Cellular activity (gene reporter): target engagement

Fluorescent quenching assay principle

Assay development in an HTS format for 2 pre-miRNA (mutant vs wt)



Compound screening at 2 doses then DRC for IC50 determination

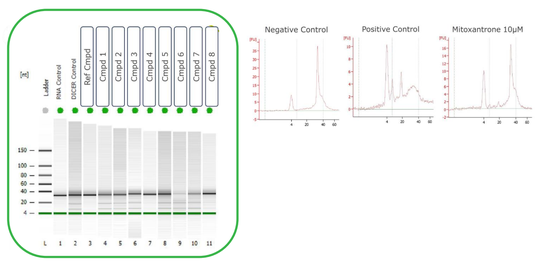


Fluorescence quenching assay

PROS
- High to medium throughput
- IC50 determination
- Low costs

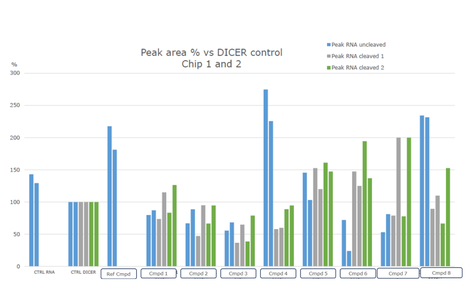
CONS
- Fluorescent cmpds interference
- No information about cleavage specificity

Analysis of cleaved miRNA
Compound screening LTS: 4 chips (11 channels/day)
Assay compatible for autofluorescent compounds



RNA control = RNA only / DICER control = RNA + DICER

Quantification



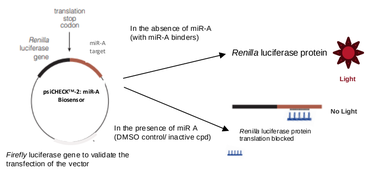
Pre-miRNA cleavage w/ Bioanalyzer

PROS
- Quantification of % of cleavage
- No interference with fluorescent cmpds
- Cleavage specificity: RNA pieces size giving insights about MoA

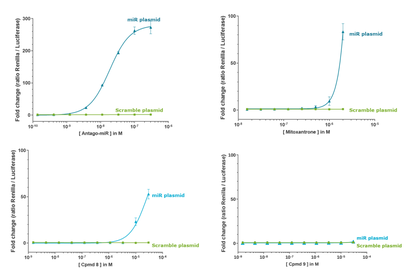
CONS
- Low throughput
- Expensive

psiCHECK2 gene reporter assay development on 5 cell lines for 2 different miRNA

HTS format

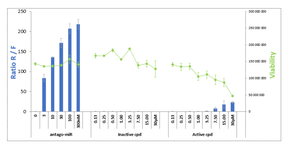


Compound screening at 2 doses than DRC for IC50 determination



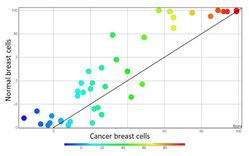
4 - Antiproliferative activity & toxicity

Cell Titer Glow proliferation assay (panel of cancer cells)



Toxicity is not linked to miRNA inhibition

Toxicity on normal cells



Off-target toxicity elucidation is ongoing

Flowchart

Compounds binding screening w/SPR
↓
Ligands characterization by affinity & kinetics w/SPR
↓
Cmpd selectivity & profiling vs Dicer & other pre-miRNAs w/SPR
↓
Inhibition of Dicer activity Fluorescence quenching (IC50)
↓
Inhibition of Dicer activity w/ bioanalyser (% cleavage)
↓
Selectivity vs pre-miRNAs
↓
Target-based cellular assay
↓
Anti-proliferative assay
↓
Toxicity

Summary

An integrated workflow for the identification of small molecule inhibitors of RNA targets is demonstrated. High-throughput screening by SPR allowed rapid identification of hit compounds and the characterization of their kinetics and affinity. Biochemical assays showed specific inhibitory activity of pre-miR maturation. Cellular assays confirmed cellular penetration, on-target activity, and anti-proliferation. The workflow was optimized to go rapidly from the hit identification to lead optimization.
Similar workflows have been developed for other RNA targets including aptamers, messenger-RNA, ribosomes, RNA-protein complexes, and trinucleotide expansion.

References

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