Development and mechanism of a small activating RNA targeting CEBPA, a novel therapeutic in clinical trials for patients with liver cancer

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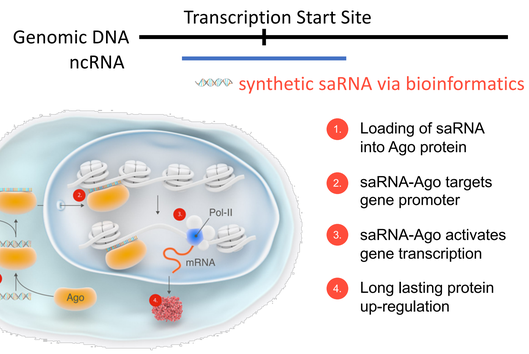
BACKGROUND

Small activating RNAs (saRNAs) are short double stranded oligonucleotides designed to upregulate their target gene by transcriptional activation. Following transfection into cells, the saRNA is loaded into Ago2 and translocates into the nucleus where it interacts specifically at the target gene leading to recruitment and activation of RNA Polymerase II (Portnoy et al., 2016; Kalantari et al., 2016). This leads to new messenger RNA production resulting in upregulation of the target protein.

Previously we published a saRNA targeting the transcription factor CCATT/enhancer binding protein alpha (CEBPA), a critical regulator of hepatocyte maturation and function which showed activity in a rat liver cancer model (Reebye et al., 2014). Here we investigate its mode of action and describe its optimization into a clinical candidate, CEBPA-51. This novel saRNA has now been tested in a variety of liver disease models, including non-alcoholic steatohepatitis, acute liver failure, and fibrosis. CEBPA-51 is currently in a Phase I clinical trial for patients with liver cancer, representing the first human study of a saRNA therapeutic.



PLATFORM

MiNA Therapeutics has developed a proprietary algorithm that identifies saRNAs recognizing non-coding RNAs upstream and downstream of the transcription start site. To date we have applied this algorithm to successfully upregulate >20 target genes in a range of cell types from human, nonhuman primate, and rodent primary and established cell lines.

REFERENCES

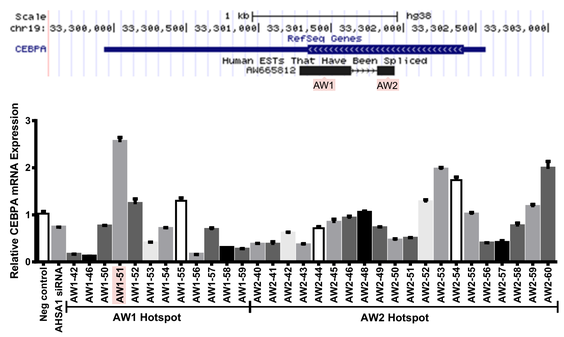
Portnoy V et al, saRNA-guided Ago2 targets the RITA complex to promoters to stimulate transcription. Cell Research 26, 320-335, 2016

Kalantari R et al, Regulation of mammalian transcription and splicing by nuclear RNAi. Nucleic Acids Research 44, 524-37, 2016

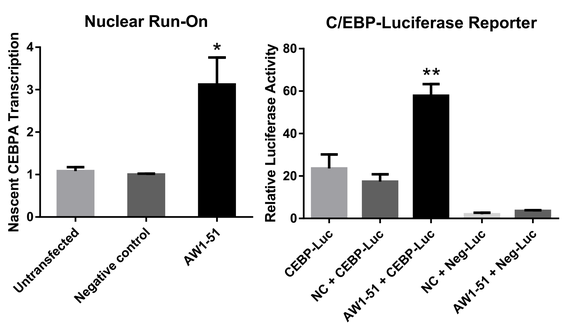
Reebye V et al, A novel RNA oligonucleotide improves liver function and inhibits liver carcinogenesis in vivo. Hepatology 59, 216-27, 2014

RESULTS

1. Nucleotide walk of potential saRNAs around bioinformatically-identified hotspots

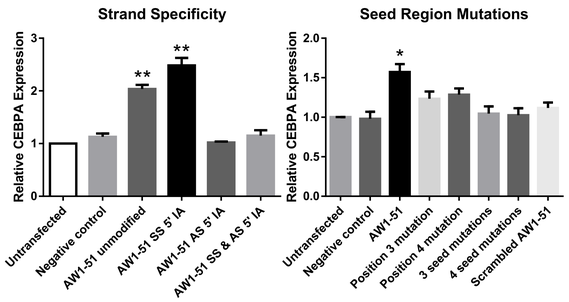


2. saRNA AW1-51 increases CEBPA mRNA transcription and protein activity



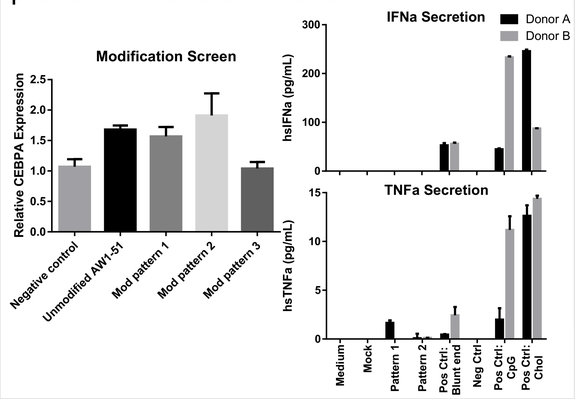
*p<0.05; **p<0.01

3. 5' inverted abasic modifications show the AS strand is the guide strand; seed region mutations demonstrate on-target activity

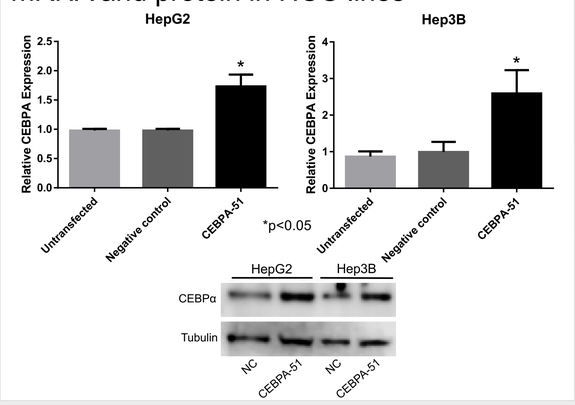


*p<0.05; **p<0.01

4. AW1-51 tolerates 2'Ome modifications to prevent immune stimulation

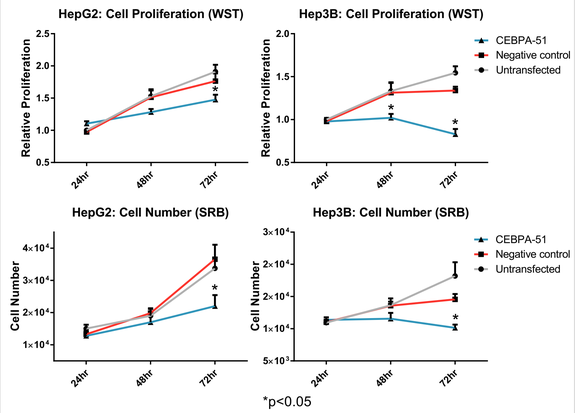


5. Modified CEBPA-51 upregulates CEBPA mRNA and protein in HCC lines



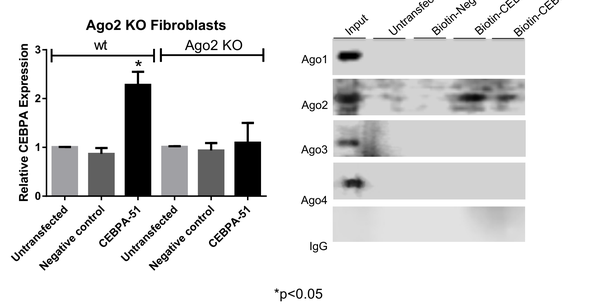
*p<0.05

6. CEBPA-51 attenuates growth in HCC lines



*p<0.05

7. Ago2 is required for saRNA activity and biotinylated CEBPA-51 Co-IPs with Ago2



*p<0.05

SUMMARY & CONCLUSIONS

- Our algorithm successfully identifies hotspots of saRNA activity, and a subsequent nucleotide walk on those hotspots determines the optimal sequence for gene activation (Fig 1).
- The increase in CEBPA mRNA is due to activation of transcription, not stabilization of existing mRNA. This increase in CEBPA mRNA results in upregulation of CEBPα protein activity (Fig 2).
- By using 5' inverted abasic modifications on each strand to block Ago2 loading, we show that the antisense strand of AW1-51 is the guide strand. Mutations introduced into the seed region of the guide strand lower activity, indicating that this is an on-target sequence-specific effect (Fig 3).
- The AW1-51 saRNA tolerates different patterns of 2'Ome modifications, and these modifications prevent stimulation by the immune system in a PBMC assay (Fig 4). The final modified version of AW1-51 is called CEBPA-51.
- CEBPA-51 upregulates CEBPA mRNA and protein in human HCC lines and inhibits cell proliferation over a 72-hour timecourse (Fig 5, 6).
- CEBPA-51 activity requires Ago2, and Ago2 co-immunoprecipitates with biotinylated CEBPA-51 (Fig 7).
- Taken together, these results indicate that the upregulation of CEBPA mRNA by CEBPA-51 is an Ago2-dependent, sequence-specific, transcriptional activation mechanism. CEBPA-51 encapsulated in the clinically validated SMARTICLES® lipid nanoparticle (MTL-CEBPA) is currently in Phase I trials for patients with liver cancer.

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