Investigating Chemically-Modified Short Activating RNAs to Increase Nuclease Stability and Gene Activation

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Abstract

Short activating RNAs (saRNAs) are short duplex RNAs that target promoters of specific genes in the nucleus of the cell. This induces gene activation, or RNA activation (RNAa) which upregulates gene expression. This activation is in direct contrast to short-interfering RNAs (siRNAs) which downregulate expression through the activation of Argonaute 2 within the RNA-induced-silencing-complex (RISC). SiRNA modifications such as 2'-OMe, 2'-F, locked nucleic acids (LNA), unlocked nucleic acids (UNA), as well as backbone modifications such as phosphorothioate (PS) have been well documented and studied. Some previous work from our lab have also shown that siRNAs containing abasic spacers linkages within the sense strand can serve as effective RNAi substrates, while providing thermal destabilization of the RNA duplex.[1,2] The goal of this study was to take some of the most well studied RISC-compatible chemical modifications for siRNAs, in order to determine their suitability for use in the saRNA pathway with the goal to increase nuclease resistance and efficacy. In this study, a library of chemically-modified saRNA modifications were synthesized and evaluated for their ability to activate gene expression. In addition, we investigated the nuclease stability of several of the modified saRNA duplexes. We have identified that an abasic carbon-based linker within the central region of the sense strand offers thermal destabilization, yet offers enhanced nuclease stability and RNA activation when compared to its parent duplex for 2 gene targets.

saRNA Pathway

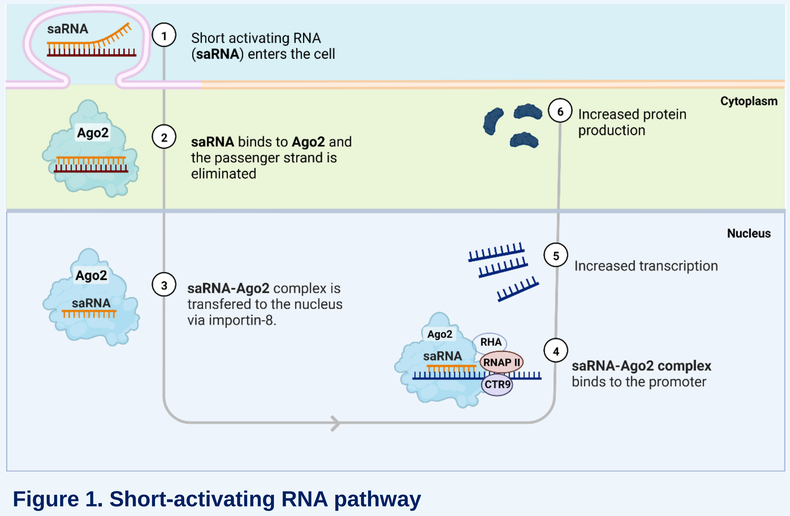


Figure 1. Short-activating RNA pathway

Previous siRNA Design

We have previously shown that abasic internal alkyl spacer linkages spanning the central region of the sense strand of siRNAs are suitable substrates for RNA interference.[1,2]

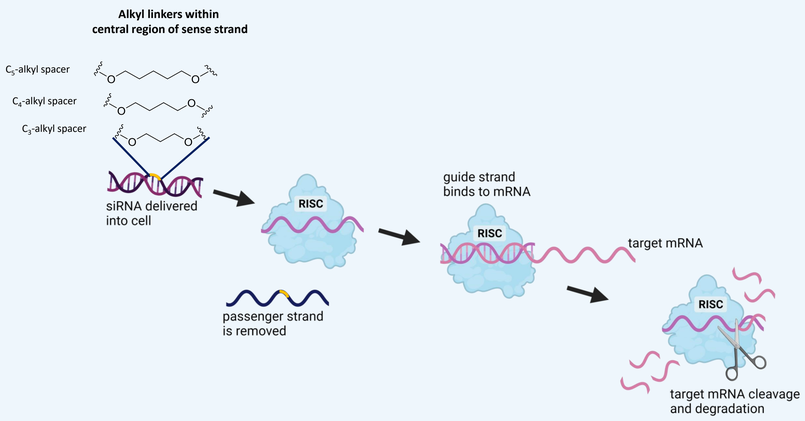
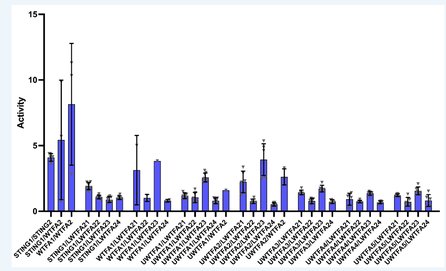


Figure 2. Carbon-based alkyl spacer linkages at the central region of the sense strand as effective siRNA substrates

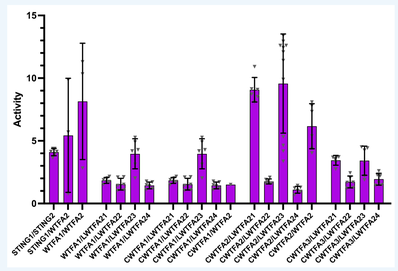
saRNA Design

Chemical composition of siRNAs plays an important role in governing efficacy, stability and thermodynamics. Although the saRNA mechanism is very different from siRNA mechanism, this study focuses on the effect of these alkyl spacer modifications, in combination with chemical modifications such as 2'-OMe, 2'-F, LNA, UNA, and backbone phosphorothioates to evaluate their use within saRNAs. SaRNAs show promise as therapeutic RNAs capable of upregulating gene transcription.[3] Thus, their efficacy, thermal stability and nuclease stability are examined.

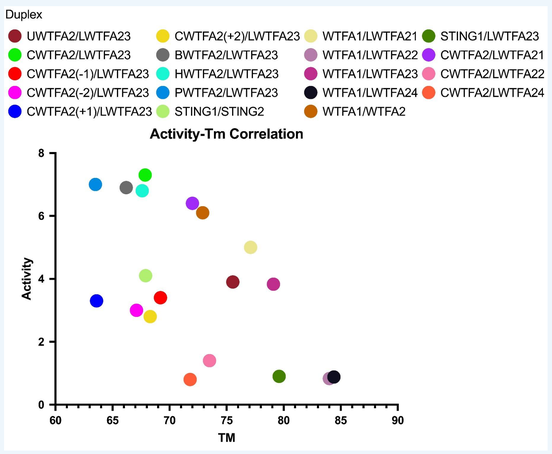
saRNA Activity



A



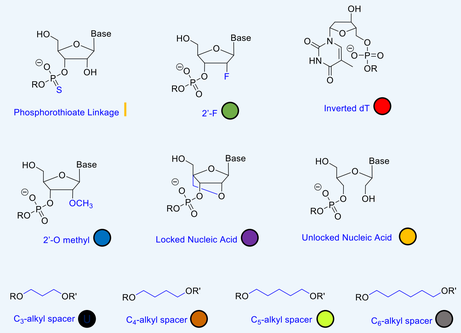
B

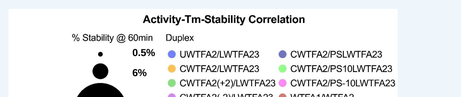


C

- UNA containing saRNAs do not activate STING significantly (**A**)
- C_3-alkyl spacer within central region enhances activity (**B**)
- Ideal T_m for duplex occurs within 63-73 ºC (**C**)
- CWTFA-2/LWTFA-23 offers T_m of 68 ºC and greatest nuclease stability(**D**)

Chemical Modifications Used in saRNA Design





D

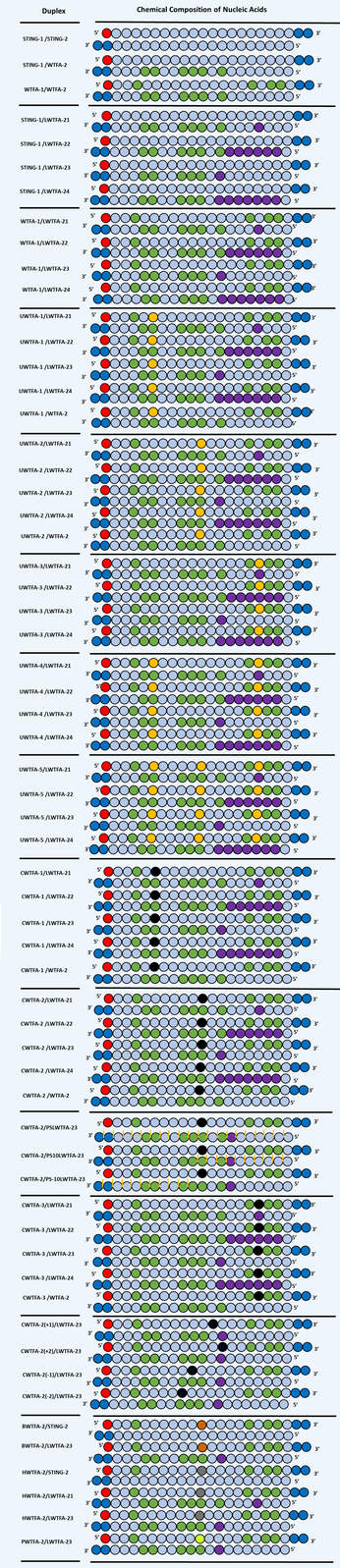
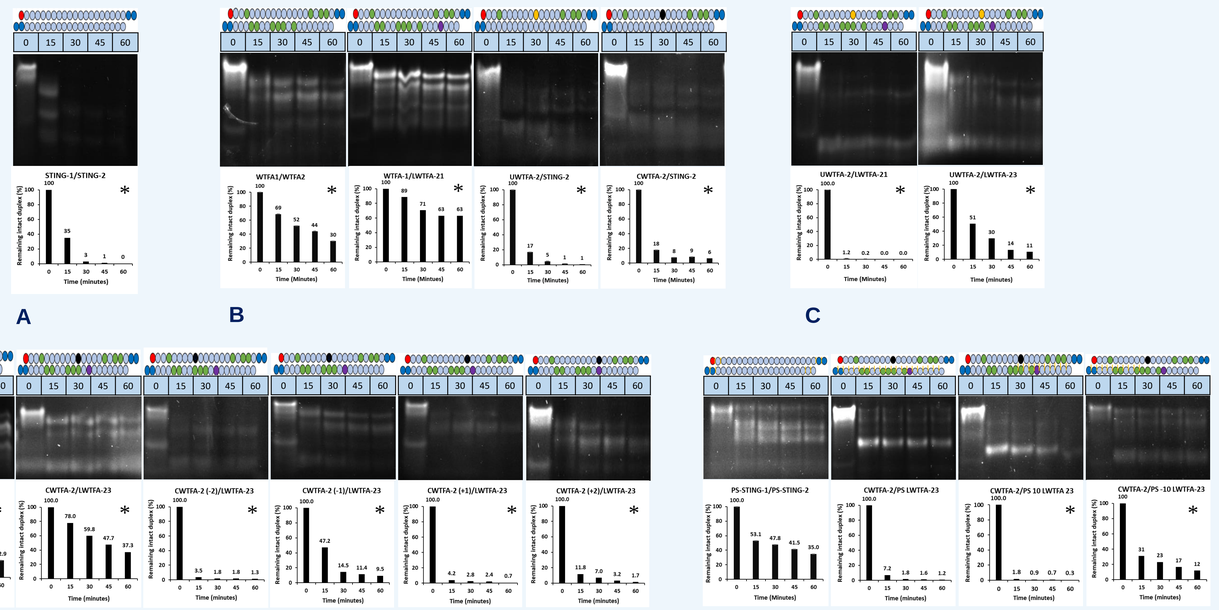


Figure 3. STING activation with saRNAs (10 nM) in A549 cells. A. Effect of chemical-modifications and unlocked nucleic acid (UNA) on activity. B. Effect of chemical-modifications and C_3-alkyl linker on activity. C. STING activation correlated with T_m of saRNA duplexes. D. STING activation correlated with T_m and stability of saRNA duplexes.

saRNA Nuclease Stability

- Unmodified canonical saRNA duplex (STING-1/STING-2) are rapidly degraded (**A**)
- Modification with some 2'-F and LNA (WTFA-1/WTFA-2) enhances stability (**B**)
- Thermally destabilizing UNA modifications with wt antisense (**B**) and modified antisense leads to degradation (**C**)
- Thermally destabilizing C_3-alkyl-based linker at positions 9 and 10 of sense strand offers greatest stability (**D**)
- 3'-Overhangs are stabilized by PS linkages (**E**)



A B C

D E

Figure 4. Effect of nuclease stability on the saRNA duplex with different chemical modifications. Stability profile of each saRNA duplex was tested using 25% FBS at different incubation time points (15, 30, 45, 60 minutes at 37 ºC) against non-treated sample at time point zero containing only saRNA and water at room temperature. Quantification of each band is represented in the perspective bar graph below the gel image representing a minimum of two bio replicates. Graphs with an asterisk (*) represent the quantification of duplexes after the overhang cleavage.

References

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