In Vitro potency of chemically modified siRNAs against TNF-α

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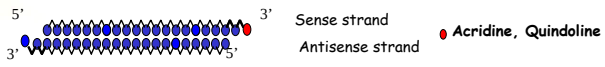
the execution of succesful RNAi experiments depends upon multiple factors, specifically: 1) Design and identification of effective and specific siRNA sites, 2) Enhancement of pharmacocinetic properties, including serum stability, and 3) efficient and specific delivery of siRNA to desired target cell types. Therefore we have design, prepared and functionality tested a variety of terminal chemical modifications to improve stability. These modified siRNAs have been tested in HeLa cells to ascertain wether they influence RNAi inducing activity. We have chosen tumor necrosis factor (TNF-a) as a target. This protein is a major mediator of apoptosis, as well as inflammation and immunity, and it has been implicated in the pathogenesis of a wide spectrum of human diseases. Therefore, the inhibition of TNF-a is of special interest as a potencial therapeutic tool.

Intercalating Agents: Acridine and Quindoline



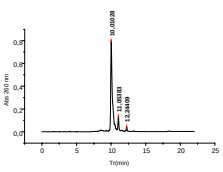


Figure 1. HPLC chromatogram obtained at 260nm after synthesis of oligonucleotide TNF-α-acridine (3' Antisense). Peak eluted at t_R=10.0 min.

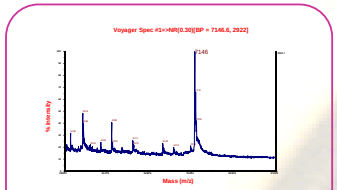


Figure 2. Matrix-assisted laser desorption ionization (MALDI) spectrum for TNF-α-acridine (3' Antisense).

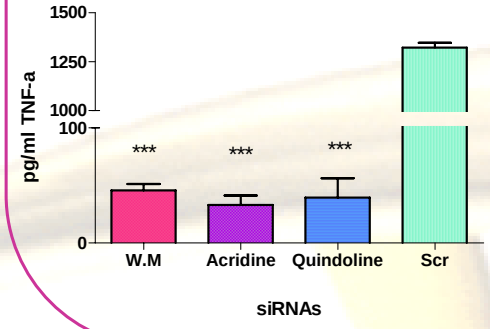
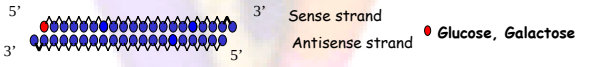


Figure 3. Potency *in vitro* of modified siRNAs with different Intercalating agents, against TNF-α using HeLa cells cotransfected with murine expressing TNF-α plasmid. Error bars represent the s.d of the mean. Statistical analysis was by ANOVA with Bonferroni post-test, one tailed. *** P<0.001 compared with scrambled siRNA. Test n=3.
W.M: Without modification
Scr: Scrambled siRNA

Sugar Ligands: Glucose and Galactose



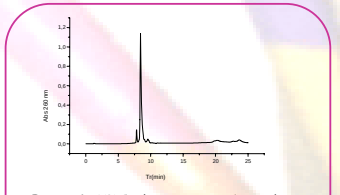


Figure 4. HPLC chromatogram obtained at 260nm after synthesis of oligonucleotide TNF-α-glucose (5'sense). Peak eluted at t_R=9.5 min.

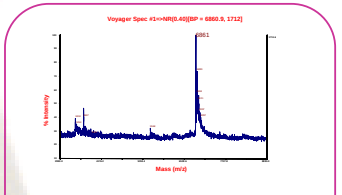


Figure 5. Matrix-assisted laser desorption ionization (MALDI) spectrum for TNF-α-glucose (5'sense).

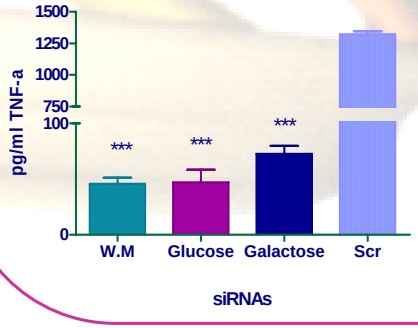


Figure 6. Potency *in vitro* of modified siRNAs with different Sugar Ligands, against TNF-α using HeLa cells cotransfected with murine expressing TNF-α plasmid. Error bars represent the s.d of the mean. Statistical analysis was by ANOVA with Bonferroni post-test, one tailed. *** P<0.001 compared with scrambled siRNA. Test n=3.
W.M: Without modification
Scr: Scrambled siRNA

Conclusions : We have demostrated that RNA carrying Intercalating Agents or Sugar Ligands can be sintetized using the stepwise approach. siRNA duplexes covalently linked to those, can be delivered to HeLa cells and these conjugates enter the RNAi pathway to silence gene expression as efficiently than unmodified siRNA duplex.

Bibliography :

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