Gene Silencing with MitoProd Circular Interfering RNA®

Introduction

RNA interference represents a powerful approach to down-regulate genes and treat diseases. Small Interfering RNA (siRNA), are used to specifically knock out disease-causing gene. However, researchers are facing stability problems, among others, having as the only alternative chemicals modifications.
MitoProd Circular Interfering RNA, ciRNA®, has been designed to bring new solutions for the development of siRNA-based drugs.
Here we demonstrate that MitoProd ciRNA® presents a resistance to RNAses, as well as an increased efficiency *in vivo* compared to siRNA.

Resistance of the ciRNA® LUC to exonuclease R treatment:

Material & Methods:
The RNA were digested with exonuclease having a 3'-5'. Exonuclease treatment was performed in the presence of 1 UI of Exonuclease R (TEBU-BIO) per microgram of RNA, for 1h at 37 °C. RNA samples were mixed with denaturation loading dye solution and 0.5 µL ethidium bromide, incubated 10 min at 65-70°C and separated by electrophoresis in native or denaturing agarose gels (1 to 2 % agarose), at a voltage of 135 V. Denaturing agarose gel (1 to 2 g agarose, 10 mL 10X MOPS buffer (0.4 MOPS, 0.1 M sodium acetate, 10 mM EDTA), 18 mL formaldehyde, 72 mL water, 1 µL ethidium bromide (10 mg/mL)) were used with running buffer containing 1X MOPS buffer (0.04 MOPS, 0.01 M sodium acetate, 1 mM EDTA). Native agarose gel (1 to 2 g agarose, 10 mL 10X TAE buffer, 90 mL water, 1 µL ethidium bromide (10 mg/mL) was used with 1X TAE buffer. Bands on gel were visualized using a UV (302 nm) transilluminator.

Results: RNAse resistance of ciRNA®

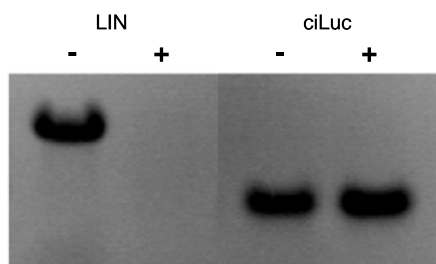

Figure 1 illustrates the resistance of the circular RNA ciLUC to exonuclease R treatment. The ciLUC was incubated with exonuclease R (1UI per microgram of RNA) for 1h at 37 °C and analyzed by electrophoresis on denaturing 1 % agarose gel (ciLuc (+)). An aliquot of the reaction mixture, not treated with exonuclease R (ciLuc (-)) and a linear RNA (LIN) were used as controls.

RNA interference activity of ciLUC

Material & Methods:
100 000 Huh-7 (human hepatocarcinoma) derived cells expressing constitutively both the *Firefly* and *Renilla* luciferase genes were grown in 24 well plates (10^5 cells/well) in DMEM medium supplemented with 20 % fetal calf serum (complete medium), for 16 h at 37 °C with 5 % CO_2. The transfection was performed by incubating 0.9 µg of RNA and 2.1 µL of DMRIE-C liposome (INVITROGEN) in 500 µL OptiMEM® (GIBCO), for 30 min at room temperature. The 500 µL transfection mixture was added to the cells rinsed with PBS and the cells were incubated for 4 h at 37 °C with 5 % CO_2. 500 µL of complete medium was added to the cells which were incubated for 48 h at 37 °C with 5 % CO_2. The cells were then rinsed three times with PBS lysed in 70 µL of lysis buffer (PROMEGA) and centrifuged 2 min at 13 000 g. The supernatant was recovered and the luciferase assay was performed on a 20 µL aliquot of the supernatant mixed with 50 µL of substrate using the Dual-Luciferase Assay System kit according to the manufacturer's instructions (PROMEGA). The light emitted by the luciferase-catalyzed chemoluminescent reaction (number of relative luciferase units or RLUs) was measured in the cells transfected with RNA and in the control cells, using a luminometer. The silencing of the luciferase gene was calculated from the ratio of RLUs in the transfected cells versus control cells.

Results: In vivo efficiency of ciRNA®

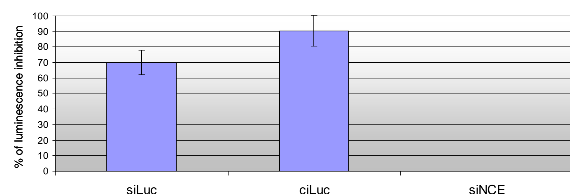

Figure 2 illustrates the RNA interference activity of the ciLUC RNA. Huh-7 cells (10^5 cells) were transfected with 900 ng of RNA. The expression of the two luciferase genes was assayed at 48 h. The values correspond to the percentage of luminescence inhibition by the RNA (ratio of RLUs in the cells transfected with RNA versus control cells (no RNA) × 100). The RNA interference activity of the circular siRNA, ciLuc, was compared to that of the corresponding siLUC from Ambion, and a non-relevant shRNA (siNCE). The plotted data are the means ± standard deviation of 4 experiments.

Conclusion

We have demonstrated that ciRNA® can resist to RNases' action, as well as being more efficient *in vivo*. With these features, coupled with others, MitoProd ciRNA® represents a new powerful tool for the development of RNA-based drugs.

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