Screening of siRNA formulations for potential leukaemia treatments

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Introduction

RUNX1/ETO is a fusion gene found in 15% of Acute Myeloid Leukaemia (AML) cases. It is required for the maintenance of the leukaemia cells. An siRNA specific to this fusion gene can destroy it without damaging normal cells but problems with the pharmacokinetics make cellular uptake a challenge.

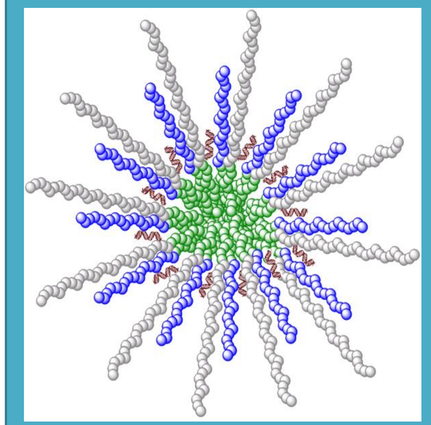

Figure 1 The polymer nanoparticle forms a mixed micelle, where the short cationic polymers (blue) bind electrostatically to the siRNA (red). The longer, neutral polymer (grey) protects the nanoparticle from the environment. [1]

A polymer nanoparticle (see Figure 1) consisting of a cationic and a neutral polymer can be loaded with the siRNA and deliver it to cells. This project focussed on finding the best combination of the two polymers and siRNA to get the best knockdown of the gene with limited toxicity.

Aims

- The aim of this project was to find an optimal formulation of polymer nanoparticle (PNP) and siRNA for the SKNO-1 cell line

Methods

Firstly, different formulations of the nanoparticle were made by dissolving the cationic and neutral polymers in water. Different ratios of the two were mixed to make PNP0 through to PNP100 where PNP100 is 100% cationic. The polymer nanoparticle was then characterised using the zetasizer to find the average size and zeta potential of the particles. Electrophoresis gels were also run to check siRNA loading onto the nanoparticles, as shown in Figure 2.

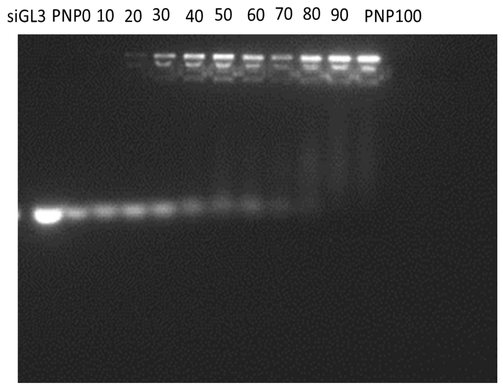

Figure 2 Electrophoresis gel showing how siRNA loading increases as the PNP becomes more cationic. The gel shown used 20uM siGL3 but this was also repeated using 6uM, 20uM and 60uM siGL3 and siAGF1 giving similar results.

A high throughput screen was then carried out by adding the different formulations at varying concentrations and luciferase to SKNO-SLIEW cells, which express the RUNX1/ETO fusion gene. The cells were incubated overnight then a luciferase assay was performed to assess the gene knockdown and toxicity. The optimum formulations were then added to larger cell volumes and RT-PCR and western blotting were used to assess the RNA and protein levels after 24 and 48 hours.

Results and Conclusions

- The particle sizes were around 90nm in diameter
- Zeta potentials were around 30mV. This positive surface charge may increase the likelihood of non-specific interactions
- Optimum formulations: 3.5uM PNP50 and 2.5uM PNP60 both with 200nM siRNA

The graph in Figure 3 shows the high throughput screen results. Any formulation showing toxicity has been excluded. The bars highlighted yellow represent the optimum formulations as they show the most knockdown

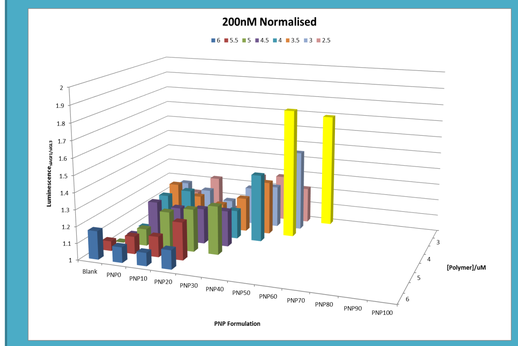

Figure 3 Normalised luciferase assay results for high throughput screen with 200nM siGL3. Toxic formulations have been removed and the optimum formulations are highlighted in yellow. These show the most knockdown.

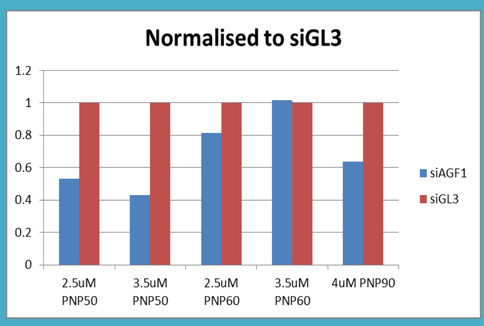

Figure 4 RT-PCR results for the optimum formulations with siAGF1 expression normalised to siGL3 expression. The 3.5uM PNP50 showed a specific gene knockdown of over 50%

A repeat of the high throughput also showed good knockdown for PNP90 at 4uM but this is thought to be an anomaly as PNP90 is expected to be toxic. The RT-PCR of the optimum formulations is shown in Figure 4. Good knockdown of the specific RNA expression was achieved for all the optimum formulations except 3.5uM PNP60. Further work is needed to improve stability in vivo and to achieve more targeted delivery to leukaemia cells.

References and Acknowledgements

[1] Omedes Pujol, M., Coleman, D., Allen, C., Heidenreich, O. and Fulton, D. (2013). Determination of key structure--activity relationships in siRNA delivery with a mixed micelle system. Journal of Controlled Release, 172(3), pp.939--945.

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