Biological optimisation of star-polymer nanoparticles for effective delivery of therapeutic siRNA to lung cancer cells

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Introduction: Lung cancer (LC) is responsible for the highest number of cancer-related deaths worldwide. Resistance to chemotherapy and molecular targeted drugs is common for this disease. Hence, there is a need to develop novel treatment strategies. Our research program has identified the microtubule protein, βIII-tubulin (TUBB3) as a therapeutic target for LC[1,2]. However, TUBB3 is considered undruggable using pharmacological inhibitors due to its high amino acid sequence and structural homology with other proteins. siRNA-based drugs offer a solution to this problem but require a delivery vehicle. The **aim** of this study was to identify the biological properties required for star-polymer nanoparticles to act as delivery vehicles for siRNA to LC cells to silence TUBB3 expression.

Methods: Star-polymer nanoparticles (star) were synthesised by RAFT[3]. Physical characterisation of star-siRNA was assessed using dynamic light scattering. A panel of human LC cell lines (H1299, Calu-6, H1975, A549) and non-tumour lung fibroblasts were used in this study. Star-siRNA delivery, release and gene silencing activity were examined using confocal microscopy, flow cytometry, qPCR and western blotting. An orthotopic syngeneic LC mouse model was used to examine star-siRNA biodistribution.

Results: Star-siRNA formed small uniform nanocomplexes (size 14.2nm ± 0.8, zeta potential 8.7mV ± 2.7, n = 3 experiments). Star when complexed to siRNA at different w/w ratios (4:1, 8:1, 16:1) were effectively internalised into LC cells. Star-siRNA complexes were pH-responsive and released siRNA from early endosomes into the cytosol of LC cells within 4h. Star-TUBB3 siRNA (16:1 w/w) was able to significantly inhibit TUBB3 protein expression in four different LC cell lines (H1229: 79% ± 8.7; Calu-6: 62% ± 7.8; H1975: 61% ± 7.2; A549: 47% ± 4.2, n = 3 experiments, $p<0.001$) 48h post-treatment. No cell toxicity was observed in non-tumour lung fibroblasts when treated with star-TUBB3 siRNA. Star-fluorescent siRNA when administered systemically to mice was internalised into growing lung tumours 24h post-injection. Star-siRNA was not toxic to mice.

Conclusions: This study demonstrates for the first time the intracellular uptake and trafficking profile of star-siRNA in LC cells. We have provided evidence that star-siRNA can silence TUBB3 expression in LC cells *in vitro* and accumulate into mouse lung tumours *in vivo.* Taken together, star-siRNA has potential to become a novel RNAi-therapeutic drug which can inhibit the expression of genes which are difficult to target using chemical drugs in lung tumours. This has potential to increase the survival of lung cancer patients.

References

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