Delivery of MicroRNA Using L-tyrosine Polyurethane Nanoparticles as a Therapy for Liver Fibrosis

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Statement of Purpose: Hepatic fibrosis typically results from excessive accumulation of extracellular matrix proteins (ECMs) due to the presence of chronic inflammation. A key mechanism implicated in the regulation of this process is the increased production of hedgehog ligands by the damaged hepatic epithelial cells.[1] In this pathway, Smoothened (Smo) becomes liberated once the hedgehog ligands bind to Patched (Ptc) and blocks intracellular kinases that phosphorylate Glioblastoma (Gli) family of proteins.[2] The accumulation and translocation of Gli to the nucleus in increases the tanscriptional activities associated with hepatic fibrosis.[2] Therefore, we hypothesize that Gil3 can be silenced by delivering microRNA into the hepatic tissue. Specifically, miR-378-3p, which targets Gil3, can be encapsulated into a biodegradable nanoparticle, uptake by the diseased liver, and used as a therapy for reversing fibrosis.[3]

Methods: L-tyrosine polyurethane nanoparticles (LTU2a-NP)[4] were encapsulated with either miR-378-3p or scrambled microRNA using water-in-oil-in-water emulsion. During the emulsion process, a copolymer of polyethylene glycol and polylactic acid was added to decorate the surface of the resulting nanoparticle with PEG.[4] Liver fibrosis was induced in 6-week-old mice (Male C57BL/6, Hyochang, Dae-gu, Korea) by i.p injections of 0.4 ml/kg of carbon tetrachloride (CCl_4) dissolved in corn oil. These mice were injected three times a week for 2 weeks. Afterwards, mice were randomly divided into three groups. The positive control group (CCl_4) received phosphate buffered saline (PBS) injections followed by additional i.p injections of 0.4 ml/kg of CCl_4 twice a week for 3 weeks. The nanoparticle control group (CCl_4+NP/NC) received an injection of LTU2a-NP loaded 6 nmol of scrambled microRNA followed by i.p. injections of CCl_4 according to the established timetable. The treated group (CCl_4+NP/M) received an injection of LTU2a-NP loaded with 6 nmol of miR-378a-3p followed by i.p. injections of CCl_4 according to the established timetable. For a negative control (NP/NC), mice were injected with corn oil without CCl_4 and LTU2a-NP was loaded with scramble microRNA according to the established timetable.

Results: Western analysis of the harvested livers shows approximately a 15-fold increase in the expression of Gil3 for CCl_4 groups as compared to the negative control (no CCl_{4a}). A single treatment with LTU2a-NP encapsulated with miR378-3a normalizes the expression Gil2 to levels, which are not significantly different from the negative control. ECM production quantified by qRT-PCR shows approximately 20, 12, 60, and 18 fold increases for mRNAs encoding for α-smooth muscle actin, vimentin, collagen, and TIMP metallopeptidase inhibitor 1, respectively. With a single treatment of LTU2a-NP encapsulated with miR378-3a, these ECM markers returned to normal levels. This treatment also normalized ALT and AST levels, classical markers associated with liver fibrosis. Finally, immunohistochemistry of liver sections shows that fibrosis can be reversed by a single nanoparticle treatment (Figure 1).

Conclusions: The delivery of LTU2a-NP encapsulated with miR-378-3p to livers induced with hepatic fibrosis decreases the transcriptional activity of Gli3. By targeting the hedgehog-signaling pathway, fibrosis can be rescued even after a single injection. Thus, LTU2a-NP nanoparticles merits further development as a therapeutic.

References:

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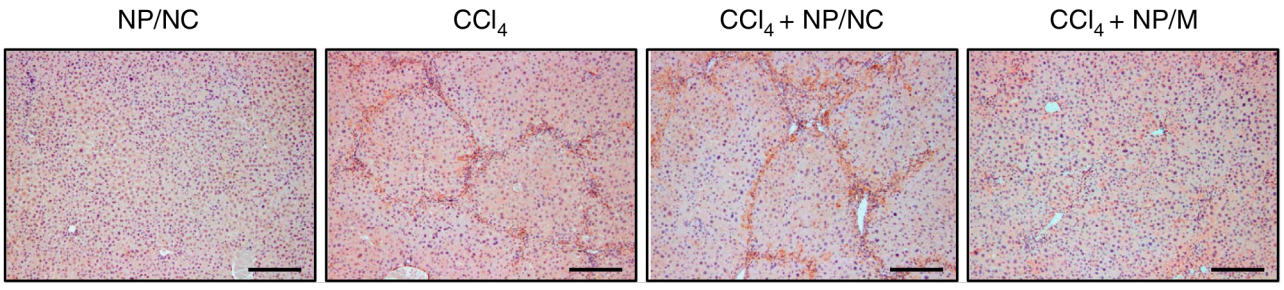

Figure 1 - Immunohistochemistry of α-smooth muscle actin for liver sections from represented. Scale bar is 100 μm.