Polyethylenimine-conjugated Arabinogalactan Based Gene Delivery Systems for Hepatocellular Carcinoma

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Statement of Purpose: Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related mortality (1). Targeting asialoglycoprotein-receptor (ASGPR), which is overexpressed on hepatocytes, is a potential strategy for targeted delivery of therapeutic agents in the treatment of HCC. Arabinogalactan (AG) from Larch tree, which is mainly composed of galactose and arabinose, was hypothesized to have the ability to target asialoglycoprotein receptor (ASGPR) overexpressed in hepatocytes (2). Due to the high biocompatibility, excellent water-solubility, and the potential of targeting ASGPR, there have been attempts to utilize AG as a drug delivery carrier for cancer treatments (3). In this study, A novel gene delivery system targeting HCC was proposed, consisting of polyethylenimine (PEI) conjugated with arabinogalactan (AGP). AG was hypothesized to have an active targeting ability towards ASGPR while PEI was expected to provide an endosome buffering effect and cationic properties. AGP was complexed with Bcl-2 siRNA by electrostatic interaction (AGP/siRNA). The AGP/siRNA complex was delivered to hepatocytes, inhibiting Bcl-2 mRNA expression and inducing apoptosis, thereby demonstrating antitumor effects.

Methods: AGP polymers were synthesized through the reductive amination of polyethylenimine (PEI) with periodate-oxidized AG. Using the newly developed one-pot synthesis method, PEI (Mw 10,000 Da) was conjugated to AG with different degrees of oxidation. Successful conjugation was confirmed by 1H NMR, FT-IR, and GPC measurements. To examine the endosomal buffering capacity of AGPs, acid-based titration was conducted. The pDNA condensation ability of AGP was evaluated using an agarose gel electrophoresis, where AGP and pDNA were mixed at various polymer-to-pDNA weight ratios (w/w) ranging from 0.1 to 1. The average particle size and zeta potential of the resulting AGP/pDNA polyplexes were measured using a Zetasizer, and their morphology at a weight ratio of 10 was further analyzed with an energy-filtering transmission electron microscope (EFTEM). The cytotoxicity of AGPs was determined through an MTT assay. Transfection efficiency was evaluated via a luciferase transgene expression assay, while the cellular uptake of AGP/pDNA polyplexes was analyzed using flow cytometry. The antitumor effect of AGPs, formed as a complex with Bcl-2 siRNA, was assessed through RT-qPCR, MTT assay, and Annexin V staining.

Results: AGPs could form positively charged nanoparticles. AGP 0.5X especially formed a stable sphere-shaped polyplex with a smooth surface and exhibited pDNA protecting ability against serum proteins. AGPs showed low cytotoxicity towards hepatocytes (HepG2, Hep3B). AGP 0.5X/pDNA polyplexes at a weight ratio of 10 exhibited the highest transfection efficiency in HepG2 cells. Meanwhile, transfection efficiency and cellular uptake of AGP 0.5X/pDNA polyplexes decreased significantly in ASGPR overexpressed cells when pre-treated with free galactose. Also, AGP0.5X/pDNA polyplexes showed superior intracellular trafficking to HepG2 compared to PEI, proving its ASGPR targeting ability (Figure 1). AGP 0.5X/Bcl-2 siRNA polyplexes successfully inhibited Bcl-2 mRNA expression and led to an antitumor effect. It was confirmed that the cell death induced by the polyplexes occurred via apoptosis

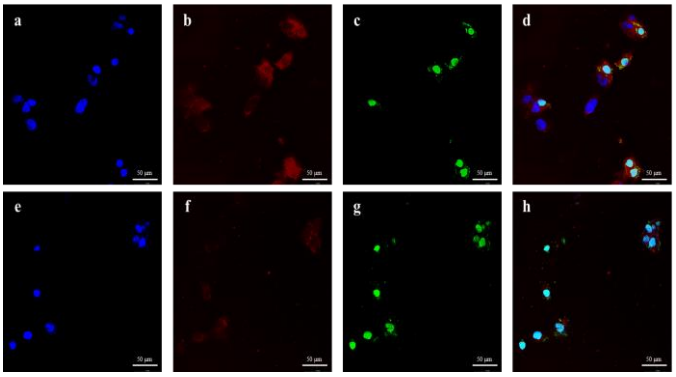

Figure 1. CLSM images of PEI25k/pDNA (wr 1) (a–d) and AGP/pDNA (e–h) after 4 h of treatment and 4 h of post-incubation in HepG2 cells. DAPI (blue) stained nuclei (a, e), Lysotracker red DND-99 (red) stained acidic organelles (b, f), YOYO-1 (green) stained pDNA (c, g), merged image (d, h).

Conclusions: In conclusion, AGPs demonstrated low cytotoxicity towards hepatocytes and effective ASGPR-targeting effect. AGP 0.5X/pDNA polyplexes showed high transfection efficiency and affinity than PEI in HepG2 cells. Additionally, AGP/siRNA complexes successfully inhibited Bcl-2 mRNA expression, inducing apoptosis and an antitumor effect. Consequently, it is concluded that AGP possesses great potential as a promising HCC-targeted gene delivery carrier. Therefore, AGP 0.5X is proposed as a potential HCC-targeted gene delivery carrier.

References: J. M. Llovet, J. Zucman-Rossi, E. Pikarsky et al. Nature Reviews Disease Primers. 2016;2:16018. **(**2) E. M. Goellner, J. Utermoehlen, R. Kramer, and B. Classen. Carbohydrate Polymers. 2011, 86(4), 1739–1744. (3) H. Wang, S. Shi, B. Bao et al. Carbohydrate Polymers. 2015;124:98–108.