Synthesis and In Vitro Assessment of Folate-targeted Polyamidoamine Dendrimer Gene Vector

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Statement of Purpose: Polycationic dendrimers have been extensively studied for gene delivery because they foster efficient internalization of DNA following endocytosis and membrane destabilization, and facilitate escape of gene/dendrimer polyplexes from endosomes and lysosomes because of proton-sponge effect (Yuan Q. Oral Oncol. 2010;7:698-704). Covalent coupling of targeting ligands to the dendrimer is a viable approach to developing efficient targeted therapeutic modalities for drug/gene delivery. Folate receptor has been shown to be frequently overexpressed in many cancers including head and neck squamous cell carcinomas (Werner ME. ACS Nano. 2011;9:8990-8998). In this work, folate-targeted polyamidoamine dendrimer nanoparticles were synthesized, characterized and investigated for folate receptor-mediated gene delivery.

Methods: Folic acid (FA) was coupled to polyamidoamine (PAMAM) dendrimer generation 4 (G4) via NHS-EDC coupling method to obtain G4-FA nanoparticles (NPs). They were further labeled with fluorescein isothiocyanate (FITC) to obtain G4-FA-FITC NPs for in vitro imaging (Fig. 1). The resulting NPs were purified by dialysis and then subjected to HPLC and ^{1}H NMR analysis.

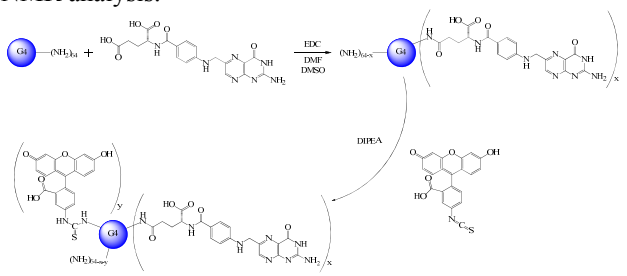

Fig. 1. Synthesis of G4-FA-FITC NP

The size and zeta potential, cytocompatibility and DNA complexation of resulting NPs were analyzed by DLS, WST-1 assay and gel retardation assay, respectively. The gene (GFP or YFP) transfection efficiency of G4-FA NPs was determined by fluorescence microscopy, flow cytometry and Western blotting. The intracellular uptake of NPs was determined using fluorescence microscopy, confocal microscopy and flow cytometry. Free FA was used to compete with G4-FA NPs in intracellular uptake and GFP transfection efficiency. G4 was used as a control for comparison.

Results: We found FRα was highly expressed in head and neck cancer cells, including HN4, HN6 and HN12 cells (data not shown). Therefore, we used FA as targeting ligand and syntheized G4-FA. HPLC anaylzis indicates the purity of resulting G4-FA NPs was more than 95% (data not shown). Compared to G4 NPs, the size of G4-FA NPs was slightly increased, still less than 10 nm in diameter; the zeta potential of G4-FA had no siginicant change, remaining positive charged (Fig. 2). The gel retardation assay indicates G4-FA could form polyplex with DNA plasmid when the weight ratio was above 1:1 (Fig. 2). G4-FA and G4 NPs were highly cytocompatible up to 10 μg/ml and 1 μg/ml in HN12 cells (date not shown). Flow cytometry, fluorescence microscopy and Western blotting results showed that G4-FA/GFP polyplexes (5:1; wt/wt) possessed higher GFP transfection efficiency in HN12 cells than G4/GFP polyplexes (Fig. 3). Flow cytometry and fluorescence microscopy results showed that intracellular uptake of G4-FA-FITC NPs was significantly inhibited in the presence of free FA (Fig. 4).

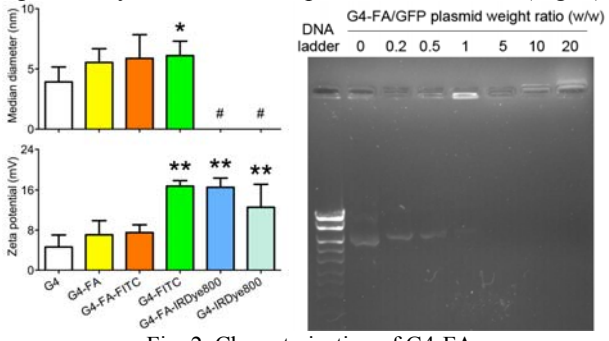

Fig. 2. Characterization of G4-FA

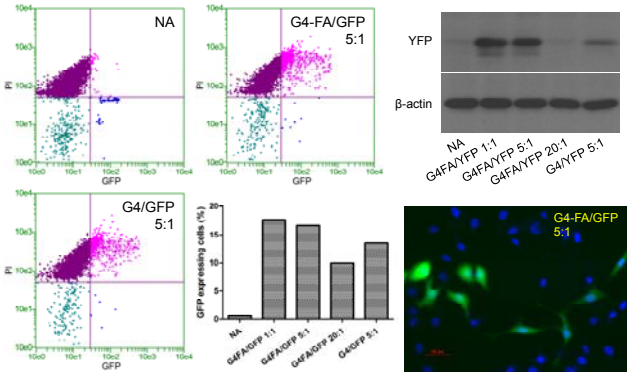

Fig. 3. Transfection efficiency of G4-FA

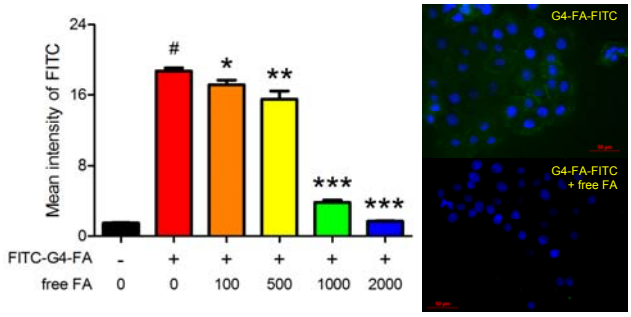

Fig. 4. Intracellular uptake of G4-FA-FITC

Conclusions: G4-FA NPs aid efficient internalization of DNA following folate receptor mediated-endocytosis. G4-FA significantly enhanced gene transfection efficiency in folate receptor overexpressed cells, compared to non-targeted dendrimer G4.

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