# Folate-targeted Polyamidoamine Dendrimer for Head and Neck Cancer Gene Therapy

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**Statement of Purpose:** Targeted delivery of small interfering RNA (siRNA) may serve as one of the most important technologies for cancer gene therapy. However, safe and efficient delivery is critical for successful development of RNA interference therapeutics (Li JM. Int J Nanomedicine. 2013;8:2101-17). Folate receptor has been shown to be frequently overexpressed in many cancers including head and neck squamous cell carcinomas (Werner ME. ACS Nano. 2011;9:8990-8). In this work, folate-targeted polyamidoamine dendrimer generation 4 (G4)/siRNA nanopolyplexes were investigated as an efficient formulation for head and neck cancer gene therapy.

**Methods:** Folate receptor expression was determined by real-time PCR and Western blotting. Folic acid (FA) was coupled to polyamidoamine dendrimer generation 4 (G4) via NHS-EDC coupling method to obtain G4-FA NPs, which was further labeled with IRDye® 800CW infrared dye (IRDye) to obtain G4-FA-IRDye NPs for in vivo imaging. The siRNA against vascular endothelial growth factor (siVEGF) was used in this work. The in vitro transfection efficiency of G4-FA/siVEGF polyplexes was determined by real-time PCR and ELISA in HN12 cells. The in vivo live imaging of G4-FA-IRDye NPs was determined using Pearl® impulse small animal imaging system, and the biodistribution of the G4-FA-IRDye NPs in different organs including heart, lung, liver, spleen, kindney and tumor at 14 d post injection was analyzed by Odyssey CLx Infrared Imaging System. The antitumor efficacy of G4-FA/siVEGF polyplexes was evaluated in HN12 head and neck tumor-bearing mouse model. The tumor tissue will be used for histology. Proliferation and angiogenesis were determined by immunohistochemical staining using antibodies to Ki-67 and CD31, respectively.

**Results:** We found FRα was highly expressed in head and neck cancer cells, including HN4, HN6 and HN12 cells (Fig. 1). Therefore, we chose 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). In vitro evaluation showed that administration of G4-FA/siVEGF significantly decreased the mRNA expression of VEGF and reduced VEGF release in HN12 cells (data not shown). At notice, G4-FA/siVEGF polyplexes were highly cytocompatible as the evidence showed that HN12 cells remained hightly viable after polyplexes transfection.

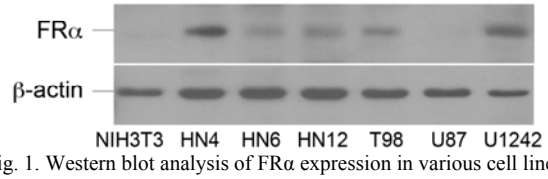


Fig. 1. Western blot analysis of FRα expression in various cell lines

In the HN12 xenograft model, intratumoral (i.t.) but not intravenous (i.v.) administration of G4-FA-IRDye or G4-IRDye significantly prolonged the NP retention time in the tumor, compared to free NIR administration (Fig. 2). The most G4-FA-IRDye NPs were only found in tumor at 14 d post i.t. administration; whereas the G4-IRDye NPs were found in both tumor and kidney. In contrast, free IRDye was found in bladder and eliminated from the body by renal clearnace within a day.

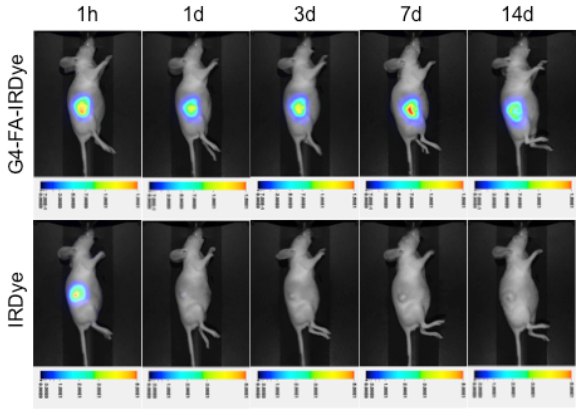


Fig. 2. Sustained retention of G4-FA via i.t injection at the tumor site.

Subsequently, one dose i.t administration of G4-FA/siVEGF polyplexes significantly delayed tumor growth and reduced tumor size in HN12 tumor-bearing nude mice, compared to PBS and G4-FA/siGFP polyplexes treated mice. At notice, i.t. administration of G4-FA/siVEGF polyplexes to the mice had no significant effect on body weight, indicating G4-FA/siVEGF polyplexes were biocompatible in vivo as well. The reduction of tumor size in G4-FA/siVEGF polyplexes-treated mice is most likely due to the lower degree of angiogenesis and the slower rate of tumor cell proliferation.

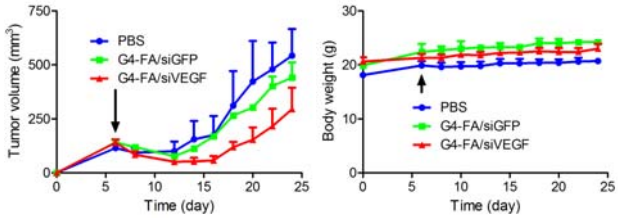


Fig. 3. Antitumor efficiency of G4-FA/siVEGF

**Conclusions:** G4-FA/siVEGF polyplexes possess good compatibility in vitro and in vivo in the investigated concentration range. The folate-targeted dendrimer, G4-FA, could significantly increase tumor accumulation and retention time, compared to non-targeted G4-IRDye NPs and free IRDye. Impressively, G4-FA/siVEGF markedly delayed tumor growth in HN12 tumor-bearing mice, indicating that folate-targeted dendrimer could serve as an efficient gene delivery system to treat head and neck cancer via folate receptor-mediated targeting strategy.

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