Quantum dot-labeled dendrimer-triglycine-EGF nanoparticles for targeted imaging and nucleic acid delivery

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Statement of Purpose: Dendrimers have emerged as the most versatile nanostructured platform for drug delivery because of their well-defined highly branched architecture and numerous surface sites for conjugation of drugs and functional moieties. In this work, we designed an EGF-containing dendrimer vector labeled with quantum dots (Q-dots) for targeted imaging and nucleic acid delivery.

Methods: EGF-conjugated dendrimers involves two steps—introducing a triglycine spacer to the dendrimer, and coupling EGF to the dendrimer via the spacer using cross-linkers NHS/EDC. Qdot® 525 ITK™ amino (PEG) quantum dots were coupled to the dendrimer via triglycine using a DSC/TEA coupling method

The molecular structure of PAMAM dendrimer conjugates G4-GGG was detected by ^{1}H-NMR. The molecular weight of G4.0-GGG was analyzed by SDS-PAGE assay. The purity of EGF-conjugated dendrimers G4.0-GGG-EGF was assessed by western blot. Tumor cell lines of HN12, HN13, NIH3T3 and NIH3T3/EGFR (SAA cells) were cultured and used to evaluate the constructed delivery system in terms of specificity and delivery efficiency. The assays including immunostaining analysis, MTT assay, and western blot were used to study targeting ability and nucleic acid delivery efficiency of this new delivery system using vimentin shRNA (shVIM) to knockdown vimentin expression as a model.

Results/Discussion: The structures of G4-GGG conjugates was characterized and confirmed by ^{1}H-NMR. SDS-PAGE analysis indicated that the surface sites of G4.0 PAMAM dendrimer was completely modified with triglycine. Western blot assay showed that G4.0-GGG-EGF conjugates had an average of one EGF molecule per dendrimer and free EGF could be completely removed from the final product. Immunostaining showed that NIH3T3 is expressing low levels of EGFR. On the other hand, NIH3T3/EGFR cells showed high expression of EGFR at the cell membrane. In the HN12 and HN13 cell lines also had strong EGFR expression in cell membrane. Various levels of EGFR in the selected cell lines indicate that these cell lines are suitable for use in our study.

Qdot-labeled EGF-conjugated dendrimers are detectable within NIH3T3/EGFR cells within 1 h, and this becomes more profound with a longer incubation period (14 h) (Figure 1). In contrast, the uptake of Qdot-labeled dendrimers lacking EGF is minimal. A similar result was also found in HN12 and HN13 cells exposed to Qdot-G4.0-GGG-EGF. Furthermore, experiments were performed in which HN13 cells were exposed to free (unconjugated) Qdots. No cellular uptake was observed, as judged by fluorescence microscopy. The result suggested that EGF-conjugated dendrimer complexes can be taken up efficiently by cells in an EGFR-dependent manner.

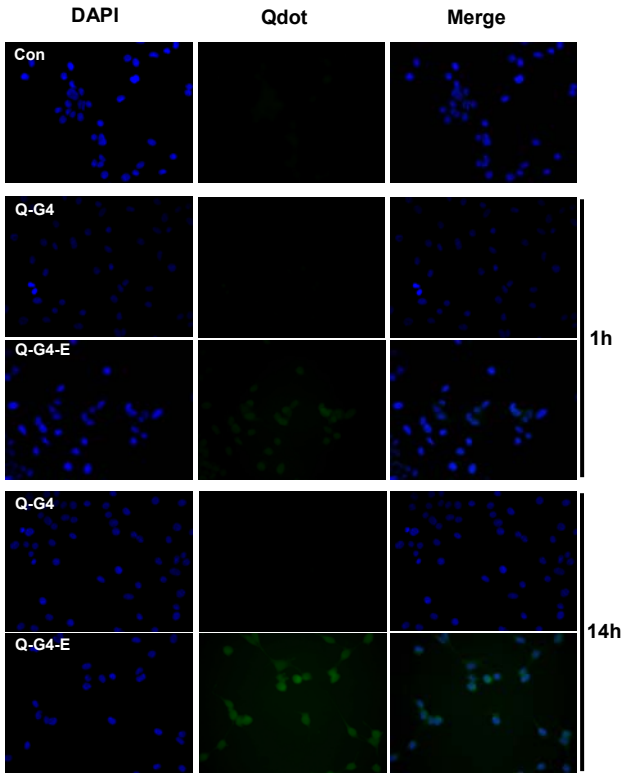

Figure 1. NIH3T3/EGFR cells were exposed to Qdot-G4.0-GGG (Q-G4) or Qdot-G4.0-GGG-EGF (Q-G4-E) nanoparticles for the indicated times, ounterstained with DAPI and imaged. Original magnification, ×400.

A significant 40% reduction in vimentin expression was found in HN12 cells treated with vimentin sh-RNA delivered by EGF-conjugated dendrimers. This result confirms the delivery efficiency of nucleic acids by EGF-conjugated dendrimers.

Conclusions: EGF-conjugated dendrimer nanoparticles may be a useful vector for targeted cell imaging and introduction of nucleic acids or drugs into cells by a growth factor-targeted mechanism.

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