Hypoxia-responsive siRNA delivery system for tumor-targeted therapy

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Purpose: To explore a novel tumor-environment-responsive modality for cancer targeting and siRNA delivery, a hypoxia-sensitive siRNA delivery system including a hypoxia sensor, 2-nitroimidazole, was constructed and investigated.

Methods: For the preparation of branched polyethylenimine-graft-hexyl-2-nitroimidazole(PCNI), a hexyl modified 2-nitroimidazole derivative was conjugated to branched polyethylenimine (bPEI, MW: 1800Da) through amide formation. siRNA could be effectively condensed by PCNI in aqueous solution to form PCNI/siRNA complexes. The size and zeta potential of the formed complexes were measured by dynamic light scattering. Polyanion competition assay was conducted by treating the complexes(N/P=2) with heparin at various concentrations, followed by agarose gel electrophoresis. Gene down-regulation efficiency of the complexes was evaluated on 4T1 cells stably expression luciferase under normoxia and hypoxia, respectively.

Results: 2-nitroimidazoles(NI) undergo selective bioreduction in hypoxic cells to form 2-aminoimidazoles(AI). The chemical structures of PCNI and PCAI were confirmed using 1H NMR spectra(Figure 1). Since NI is poorly soluble in water, the amphiphilic PCNI conjugates are able to form micelle-like aggregates by self-assembly in aqueous medium, this micellar structure contributes to the stability of siRNA in blood circulation. However, in the hypoxic tumor cells, NI is converted to AI, which is highly water-soluble, leading to destruction of the micellar structure. The particle size of the complexes was increased with the structural change(Figure 2, A), and siRNA molecules tended to be displaced by heparin at lower concentration in the formed loose structure than in the PCNI complexes(Figure 2, B). These phenomena revealed that PCNI/siRNA complexes could selectively release the siRNA in the hypoxic cytoplasm. In vitro gene transfection, compared with naked siRNA, Lipofectamin 2000, and bPEI, the luciferase expression of PCNI/siRNA Luc complexes treated 4T1 Luc cells under hypoxia was significantly lower than that under normoxia(Figure 2, C).

Conclusions: Hypoxia is a feature of most tumors. Hypoxia-responsive PCNI/siRNA complexes was stable in physiological conditions and could selectively release siRNA under hypoxic conditions. This novel siRNA delivery system showed improved tumor targeting and antitumor activity, having great potential for cancer treatment.

References: