Development and stability comparison of targeted therapeutic nanomolecules of aptamer-miRNA conjugates using two methods of conjugation

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Abstract

Introduction: An important issue in cancer therapy is the achievement of desired therapeutic response with the least adverse effects. To achieve this goal, targeted drug delivery systems were developed. Aptamers, mainly DNA/RNA aptamers, are the attractive affinity ligands for the cancer cell surface specific antigens. Besides, microRNAs are another type of therapeutic and diagnostic oligonucleotides that have been recently studied in various cancers. miRNAs are small double stranded RNAs with important roles in cell regulatory pathways. Profile changes of miRNAs can result in cancer development. External addition of miRNAs or their elimination using antagomiR can lead to the efficient treatment of related disease. Targeted delivery of therapeutic agents to the site of action with less adverse effects is the most challenging issue in anticancer chemotherapeutic agents as well as miRNA therapy. In addition, miRNAs stability in biological systems can be improved by targeting strategy. In this study, a cancer specific aptamer (anti-nucleolin aptamer) and a functional miRNA in cell growth and proliferation (miRNAlet-7d) were used in the development of targeted nano-molecules as an efficient anti-proliferative agent for cancer cells.

Methods and Results: Sequences of A1411 aptamer and miRNA let-7d were extracted from related databanks and were chemically synthesized with amine and thiol modification in the 3' terminals or with a 17 nucleotides sticky extension at 3' terminal. The sequences were conjugated covalently using $SM(PEG)_2$ hetero-bi-functional cross-linker or un-covalently by annealing the sticky ends. Conjugation was confirmed using polyacrylamide gel electrophoresis 15%. The serum stability of these two types of conjugates were evaluated using up to 48 h incubation of conjugates in human serum (AB^+). Stability of covalent conjugate using $SM(PEG)_2$ linker was at least two hours more than the un-covalent one.

Conclusions: Remarkable advantages of this nano-molecule were targeted and relative stable delivery of miRNA as the therapeutic agent with probable synergistic effect of two oligonucleotides of miRNA and aptamer in the proliferation inhibition of cancer cells.

Key words: Anti-nucleolin aptamer, miRNA let-7d, Conjugation, Stability

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