Aptamer-mediated selective and modulable siRNA delivery

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Résumé

Small interfering RNAs (siRNAs) are specific and effective molecules for gene silencing. However, their use is limited by poor cell penetration due to their negative charge, size and hydrophilicity. Active targeting delivery, the conjugation of siRNAs to ligands targeting cell-surface receptors, is a promising approach to overcome these barriers. Aptamers appear to be interesting candidates since they are single-stranded DNA or RNA with high affinity and selectivity for a specific target, such as cell-surface receptors. Thus, we aim to develop selective and modulable vehicles associating an siRNA with one or more aptamers called mono- or multivalent aptamer-siRNA chimeras (AsiCs). As a proof of concept, we are using an siRNA PGL3 anti-luciferase, combined with the E07 RNA aptamer anti-human epidermal growth factor receptor (EGFR) identified by Li *et al.* (2011).

First, to investigate the possibility of combining RNA and DNA aptamers in one vehicle, we created AsiCs formed thanks to an RNA-RNA or RNA-DNA sticky bridge. In the RNA-RNA AsiC, the sense strand of siRNA and the aptamer are elongated with RNA complementary sequences to form the sticky bridge. In the RNA-DNA AsiC, the nature of aptamer sticky bridge is change to DNA to mimic the addition of DNA aptamer. Our preliminary results show a similar assembly. Furthermore, a functional assay was performed with an RNA-RNA AsiC on two glioblastoma cell lines, modified to express luciferase: U87 EGFR LUC (EGFR-positive cell line) and LN319 LUC (EGFR-negative cell line). Luciferase expression was significantly decreased using the RNA-RNA AsiC on U87 EGFR LUC compared to non-treated cells. Second, we have designed an innovative homomultivalent AsiC, which combines one siRNA with two E07 aptamers (E07). The various elements contain hybridization sequences which enable controlled self-assembly. So far, we have predicted their secondary structure thanks to prediction software (RNAfold and predict1), and checked the vehicle assembly.

Our preliminary results of RNA-RNA and RNA-DNA AsiCs are encouraging and confirm the feasibility to combine DNA and RNA aptamers in a multivalent AsiC, a new siRNA active delivery tool with great potential. Thanks to their versatile nature, the number, position, and type of aptamers (DNA or RNA, homo- or heteromultivalent) could be easily

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changed. As perspectives, we wish to (1) deepen the characterization of AsiCs (stability and affinity), (2) perform functional assays with RNA-DNA AsiC and multivalent vehicles, and (3) study their intracellular trafficking by bioimaging. We will use either a pH-ratiometric probe developed by our collaborator or a FRET-based approach to track the AsiC during endocytosis.

Mots-Clés: siRNA, aptamer, active targeting delivery, innovative delivery tool, multivalent conjugate, cell surface receptor