A non-covalent peptide-based strategy for siRNA delivery

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Abstract
The major obstacle to clinical development of siRNAs (short interfering RNAs), like for most of the nucleic-acid-based strategies, is their poor cellular uptake and bioavailability. Although several viral and non-viral strategies have been proposed to improve siRNA delivery, their applications in vivo remain a major challenge. We have developed a new strategy, based on a short amphipathic peptide, MPG, that is able to form stable nanoparticles with siRNA. MPG-based particles enter the cell independently of the endosomal pathway and can efficiently deliver siRNA in a fully biologically active form into a variety of cell lines and in vivo. This short review will discuss the mechanism and the potency of the MPG strategy for siRNA delivery both in vitro and in vivo.

Challenges in siRNA (short interfering RNA) delivery
RNAi (RNA interference) constitutes a ubiquitous mechanism in eukaryotic cells to silence the post-transcriptional expression of genes that control fundamental events in the cells, as well as to protect cells from viral infections [1,2]. As every cell contains the RNAi machinery and potentially any gene can be targeted with high specificity, the possibility of using this endogenous mechanism of gene silencing has given great hope for numerous therapies [3,4]. The major obstacle to clinical development of siRNAs, like for most of the nucleic-acid-based strategies, is their poor cellular uptake and bioavailability. Several viral and non-viral strategies have been proposed to improve the delivery of either siRNAs expressing vectors or synthetic siRNAs [3,4]. Although the delivery of siRNA into cultured cells can be achieved by most delivery agents, their applications on primary cell lines and in vivo remain a major challenge. In vivo delivery of siRNA, with the exception of mucosal tissues where siRNA uptake is extremely efficient, remains a major issue for the development of siRNA as small-molecule drugs [4].

CPPs (cell-penetrating peptides) have been shown to efficiently improve intracellular delivery of various biologically active molecules into cells both in vitro and in vivo [5,6]. CPPs are able to overcome both extracellular and intracellular limitations. They can trigger the movement of a cargo across the cell membrane into the cytoplasm of the cells and improve its intracellular routing, thereby facilitate the interaction with the target. Most of the CPPs are covalently attached to their cargo by either chemical cross-linking or cloning. Conjugation strategy with either Transportan [7] or Penetratin [8] has been successfully applied to improve the delivery of siRNA into cultured cells. We have developed a non-covalent peptide-based strategy for the delivery of molecules into mammalian cells, using short amphipathic peptides that form stable complexes with cargoes [8,9]. The short amphipathic peptide MPG efficiently delivers nucleic acids, including siRNA, in a fully biologically active form into a variety of cell lines and in vivo [10,11]. This short review will discuss the mechanism and the application of the MPG strategy for siRNA delivery both in vitro and in vivo.

Design and structure of MPG
MPG is a 27-residue-long primary amphipathic peptide (acetyl-GALFLGFLGAAGSTMGAWSQPKKKRKV-cysteamide), which contains three distinct domains: a N-terminal hydrophobic motif (GALFLGFLGAAGSTMGA) derived from the fusion sequence of the HIV-1 gp41 (glycoprotein 41), required for interaction with the lipid moiety of the cell membrane and cellular uptake, a hydrophilic domain derived from the NLS (nuclear localization sequence) of SV40 (simian virus 40) large T-antigen (KKKKRV) involved in the interactions with nucleic acids and intracellular trafficking of the cargo, and a linker domain (WSQP), which improves the flexibility and integrity of the hydrophobic and the hydrophilic domains. MPG carries a cysteamide group at its C-terminus, which is essential for both cell entry and stabilization of the complexes with siRNA [9,10]. MPG, originally designed to improve the cellular uptake of oligonucleotide and plasmid, was then optimized for the delivery of siRNA. A single mutation on the second lysine residue of the NLS to serine (MPG^NLS GALFLGFLGAAGSTMGAWSQPKKKRKV), was shown to abolish the nuclear translocation and facilitate a rapid release of the siRNA in the cytoplasm [10].
**Figure 1** | Mechanism of cellular uptake of MPG–siRNA complexes

(1) Formation of the MPG–siRNA complexes through electrostatic and hydrophobic interactions. (2,3) Interaction of the MPG–siRNA complex with the cell surface involving electrostatic contacts with the phospholipid headgroups. (4) Insertion of the complex into the membrane, associated with formation of transient transmembrane \( \beta \)-structures. (5) Release of the MPG–siRNA complex into the cytoplasm or its nuclear targeting.

**MPG forms stable complexes with siRNA**

The MPG peptide associates rapidly in solution with siRNA, initial contact occurs through electrostatic interactions involving the hydrophilic lysine-rich domain independently of specific sequences, followed by peptide–peptide interactions involving the gp41 hydrophobic domain, thus generating stable MPG–siRNA nanoparticles with size of approx. 200 nm diameter [10]. Characterization of MPG–siRNA complexes either by fluorescence spectroscopy, gel mobility-shift assay or light scattering demonstrated that MPG exhibits high affinity in the nanomolar range for siRNA and that approx. 10–20 peptide molecules are required to form a highly stable nanoparticle [10]. The presence of a peptide-based nanostructure associated to the siRNA dramatically improved its stability inside the cell and significantly protected it from degradation [9,10].

**Cellular uptake mechanism of MPG–siRNA particles**

More recently, the cellular uptake mechanism of CPPs has been completely revised and shown to be essentially associated with the endosomal pathway [12]. However, as for most CPPs, evidence for several cell-entry routes have been reported, some of which are independent of the endosomal pathway [6], so it is essential to identify the one leading to a biological response.

An important criterion to be considered is the structural requirements for the cellular uptake of CPPs. Therefore the mechanism of MPG has been investigated from a structural point of view and we demonstrated that the outer part of the ‘carrier-based nanoparticle’ with the siRNA plays a key role in the interactions with the membrane. MPG strongly interacts with phospholipids, through their hydrophobic fusion sequence, which then adopted a \( \beta \)-structure required for insertion of the peptide into the membrane [13]. On the basis of both structural and biological investigations, a four-step mechanism has been proposed (Figure 1): (i) formation of the MPG–siRNA complexes through electrostatic and hydrophobic interactions, (ii) interaction of the MPG–siRNA complex with the cell surface involving electrostatic contacts with the phospholipid headgroups, (iii) insertion of the complex into the membrane, associated with formation of transient transmembrane \( \beta \)-structures that temporarily affect the cell membrane organization, and (iv), finally, the release of MPG–siRNA complex into the cytoplasm or its nuclear targeting [6,10,13].

In order to avoid any mechanism based on artefact, it is essential to correlate the uptake pathway of the CPP to the biological response of the cargo. The uptake mechanism of MPG–siRNA particles was investigated following the level of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) silencing. Experiments performed in the presence of several inhibitors of the endosomal pathway and after energy depletion, demonstrated that the efficiency of MPG uptake is directly correlated to the size of MPG–siRNA nanoparticle and that the mechanism through which MPG delivers active macromolecules is independent of the endosomal pathway and is mediated by the membrane potential [10,11].
Application of MPG for siRNA delivery

The MPG strategy has been used successfully for the delivery of siRNA into a large panel of cell lines including adherent and suspension cell lines, as well as primary and embryonic stem cells which cannot be transfected using other non-viral approaches [11]. The mechanism through which MPG delivers active macromolecules allows the control of the release of the cargo in the appropriate target subcellular compartment. Therefore, by tampering with the NLS sequence of MPG, delivery between the nucleus and the cytoplasm can be discriminated, and MPG containing the NLS efficiently was reported to deliver promoter-directed siRNA into the nucleus to inhibit transcription [14]. MPG has also been successfully applied for delivery of siRNA in vivo into mouse blastocytes [15] and by topical and systemic injections. That MPG forms highly stable nanoparticles with siRNA with a low degradation rate, which can be easily functionalized for specific targeting, are major advantages for in vivo siRNA delivery, in comparison with the covalent CPP technology.

Conclusions

siRNAs are promising small-molecule drugs for novel therapeutic strategy, but, like most nucleic-acid-based therapies, they are limited by the poor cellular uptake and bioavailability. With the aims of improving siRNA cellular uptake, we have developed a strategy, based on a short amphipathic peptide, which forms stable nanoparticle with cargoes. MPG technology has been successfully applied to the delivery of siRNA in primary cell lines and in vivo. Although MPG technology is still in its early days and needs to be optimized for systematic in vivo applications, it is already a powerful tool for basic research and will have a major impact on the use of siRNA for future therapies.

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References


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