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A genetically synthetic protein-based cationic polymer for siRNA delivery

Ying Liu [a], Zhilong Jia [b], Liwen Li [a], Fulin Chen [a,*]

[a] The College of Life Sciences, Northwest University, Xi'an, Shaanxi 710069, China
[b] Department of Chemistry and Biology, College of Science, National University of Defense Technology, Changsha, Hunan 410073, China

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SUMMARY

In recent years, a large number of researchers have paid much attention on small interfering RNA (siRNA) after the advent of RNA interference technology, which has been harnessed as an efficient way of sequence-specific gene silencing in gene therapy, enables elucidation of gene functions, and the identification of new drug targets. Despite tremendous progress has been made in novel delivery systems and vectors via formulation of polyplexes and conjugations, such as cationic polymers (LPEI, BPEI), cationic liposome (DOTAP), peptides (CPP), unmet needs still exist. Many cationic agents used for condensing siRNA often exhibits severe cytotoxicity, which limits clinical applications, and is obliged to be handled. Thus great interest in searching for novel and sophisticated polymeric vectors has been spurred. Herein we proposed a genetically synthetic protein-based polymer, which is also referred to as elastin-like polypeptides (ELPs) excerpted from human tropoelastin highly repetitive sequence, Val-Pro-Gly-Xaa-Gly, where the "guest residue" Xaa is any amino acid except Pro. Thus, if we alternate the "guest residue" Xaa to Lys or Arg, to a significant extent, it can emerge as a powerful cationic polymer for siRNA delivery carrier, and hopefully it will be put into practice in the near future.

Background

ELPs have a characteristic thermal property, transition temperature (T_t), which leads to the phase transition. ELPs are soluble in aqueous solution below the inverse transition temperature (T_t, also known as the lower critical solution temperature or LCST). However, when the temperature is raised above the T_t, they undergo a sharp (~2 °C range) phase transition, hydrophobically collapse and aggregate into more 'ragid' form [1]. The uncharged thermally responsive elastin-like polypeptides (ELPs) as the drug carrier have ever been widely used in targeted drug delivery and controlled release of radionuclides, chemotherapeutics and biomolecular therapeutics to tumors to enhance the localization of ELP-drug conjugates within a solid tumor that is heated by regional hyperthermia if their transition temperature (T_t) is between body temperature and the temperature in a locally heated region, which must be lower than 42 °C [2]. When comes to charged pentapeptide repeats of ELPs, consisting of guest residues such as lysine, glutamic acid, and aspartic acid, commonly it has a much greater T_t than body T(>100 °C). However, when complexed with an ionic, oppositely-charged compound, their T_t reduced amazingly with increasing ion concentration. These ELPs then transitioned at lower temperatures and entrapped the compounds. This phenomenon has ever been considered as a method for encapsulating and releasing ionic drugs from a biodegradable hydrogel [3]. From all the above, let's boldly vision whether can we design a scenario for siRNA delivery?

Hypothesis

According to present researches, we design the following new model of siRNA delivery system. This model is made up of siRNA, positive-charged ELP, and a cell penetrating peptide (CPP), penetratin, and a fusogenic peptide, KALA. Different parts of the model exert their own special effects in the course of siRNA delivery to tumors and other target sites of action.

Exploiting genetically engineered morphology, penetratin and KALA are firstly fused to the positive-charged ELP containing the 'guest residue' Lys or Arg. According to our design, Lys would be better and can be explained in the next section.

Furthermore, the cationic polypeptide polymers can interact with siRNA through electrostatic effect and covering its negative charges to form a cell-penetrating complex. The cell-penetrating peptide, penetratin can cross the plasma membrane and internalize into cells together with other fractions by endocytosis pathway [4]. KALA is well known for its destabilization of the endosomal membrane, and thus, herein we adopt it as a core forming agent to facilitate the endosomal escape process [5].

Then after escaping from the endosome, under physiological conditions in cytoplasm, ELP containing Lys residue will undergo self-association in a process referred to as coacervation and

* Corresponding author. Address: The College of Life Sciences, Northwest University, No. 229, Taibai North Road, Beilin District, Xi'an, Shaanxi 710069, China. Tel.: +86 13572534880, +86 29 88302634; fax: +86 29 88303534.
E-mail address: cflfx@163.net (F. Chen).

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cross-links into the insoluble protein by selective oxidation of lysyl oxidase at Lys residues [6]. The resulting complex can provide further excellent protection of siRNA from being attacked by intracellular nucleases, which could significantly enhance biological half-life with a concomitant increase of delivery efficiency to the target site of action while maintaining sufficient gene silencing activity.

In addition, the cationic polypeptide polymer has a good biocompatibility, which is quickly removed out of body with metabolism after the siRNA is effectively delivered to the destination. The released siRNA continually exerts its therapeutic or/and specific gene silencing effect in the target site.

Evaluation of the hypothesis

For therapeutic applications of small interfering RNA (siRNA), serum stability under normal physiological conditions, enhanced cellular uptake, and facile endosome escape are vital to design carriers. Many advantages of ELPs make them to be candidate of siRNA delivery carrier. Firstly, as described above, the cross-linking of ELPs at Lys residue could largely reduce the degradation of siRNA resulted from intracellular nucleases in cytoplasm before achieving the target site of action. Secondly, free ELPs did not display any intrinsic cytotoxicity [7], which is seriously considered in the process of designing siRNA delivery carrier by vast researchers. Furthermore, ELPs are made up of amino acids that can be degraded into non-toxic metabolites unlike some synthetic polymers [3]. Finally, because ELPs are highly repetitive sequence polypeptide, it's easy to obtain multimer gene segments of desired size by genetical synthesis approach. Simply by increasing MW, it would prolong the plasma half-life of siRNA [3].

Moreover, CPPs were first used for the delivery of proteins that were genetically fused to the CPPs. Subsequently, CPPs have been used for the delivery of many types of cargo molecules. They are proposed to interact with the anionic surface of the plasma membrane and enhance internalization of the peptides [4]. They undoubtedly have the ability to enter cells through uptake in the endocytotic pathway or by crossing the plasma membrane directly.

Briefly, this cationic-charged ELP acts as a promising carrier for siRNA drug delivery by ionic interaction with siRNA. With the help of CPPs and KALA, siRNA can be guided precisely and effectively to arrive at the intended target site of action. In summary, this new cationic polymer material with CPPs and KALA conjugation will be a powerful approach for siRNA drug delivery system.

Conflicts of interest statement

None declared.

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