Acta Biomaterialia 59 (2017) 221–233

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Functionalized bioengineered spider silk spheres improve nuclease resistance and activity of oligonucleotide therapeutics providing a strategy for cancer treatment

Anna Karolina Kozlowska [a], Anna Florczak [a,b], Maciej Smialek [b,1], Ewelina Dondajewska [a], Andrzej Mackiewicz [a,b], Marcin Kortylewski [c], Hanna Dams-Kozlowska [a,b,*]

[a] Chair of Medical Biotechnology, Poznan University of Medical Sciences, 61-701 Poznan, Poland
[b] Department of Diagnostics and Cancer Immunology, Greater Poland Cancer Centre, 61-866 Poznan, Poland
[c] Department of Immuno-Oncology, Beckman Research Institute, City of Hope National Medical Center, Duarte, CA 91010, USA

ARTICLE INFO

Article history:
Received 23 March 2017
Received in revised form 6 July 2017
Accepted 6 July 2017
Available online 8 July 2017

Keywords:
Bioengineered spider silk spheres
CpG-siRNA
STAT3
Targeted delivery
Cancer immunotherapy

ABSTRACT

Cell-selective delivery and sensitivity to serum nucleases remain major hurdles to the clinical application of RNA-based oligonucleotide therapeutics, such as siRNA. Spider silk shows great potential as a biomaterial due to its biocompatibility and biodegradability. Self-assembling properties of silk proteins allow for processing into several different morphologies such as fibers, scaffolds, films, hydrogels, capsules and spheres. Moreover, bioengineering of spider silk protein sequences can functionalize silk by adding peptide moieties with specific features including binding or cell recognition domains.

We demonstrated that modification of silk protein by adding the nucleic acid binding domain enabled the development of a novel oligonucleotide delivery system that can be utilized to improve pharmacokinetics of RNA-based therapeutics, such as CpG-siRNA. The MS2 bioengineered silk was functionalized with poly-lysine domain (KN) to generate hybrid silk MS2KN. CpG-siRNA efficiently bound to MS2KN in contrary to control MS2. Both MS2KN complexes and spheres protected CpG-siRNA from degradation by serum nucleases. CpG-siRNA molecules encapsulated into MS2KN spheres were efficiently internalized and processed by TLR9-positive macrophages. Importantly, CpG-*STAT3*siRNA loaded in silk spheres showed delayed and extended target gene silencing compared to naked oligonucleotides. The prolonged *Stat3* silencing resulted in the more pronounced downregulation of interleukin 6 (IL-6), a proinflammatory cytokine and upstream activator of STAT3, which limits the efficacy of TLR9 immunostimulation.

Our results demonstrate the feasibility of using spider silk spheres as a carrier of therapeutic nucleic acids. Moreover, the modified kinetic and activity of the CpG-*STAT3*siRNA embedded into silk spheres is likely to improve immunotherapeutic effects *in vivo*.

Statement of Significance

We demonstrated that modification of silk protein by adding the nucleic acid binding domain enabled the development of a novel oligonucleotide delivery system that can be utilized to improve pharmacokinetics of RNA-based therapeutics. Although, the siRNA constructs have already given very promising results in the cancer therapy, the *in vivo* application of RNA-based oligonucleotide therapeutics still is limited due to their sensitivity to serum nucleases and some toxicity. We propose a carrier for RNA-based therapeutics that is made of bioengineered spider silk. We showed that functionalized bioengineered spider silk spheres not only protected RNA-based therapeutics from degradation by serum nucleases, but what is more important the embedding of siRNA into silk spheres delayed and extended target gene silencing compared with naked oligonucleotides. Moreover, we showed that plain silk spheres did not have unspecific effect on target gene levels proving not only to be non-cytotoxic but also very neutral vehicles in terms of TLR9/STAT3 activation in macrophages. We demonstrated advantages of novel delivery technology in safety and efficacy comparing with delivery of naked CpG-*STAT3*siRNA therapeutics.

* Corresponding author at: Department of Cancer Diagnostics and Immunology, Greater Poland Cancer Center, 15 Garbary St., 61-866 Poznan, Poland.
E-mail address: hanna.dams-kozlowska@wco.pl (H. Dams-Kozlowska).
[1] Present address: Institute of Human Genetics, Polish Academy of Sciences, 60-479 Poznan, Poland.

http://dx.doi.org/10.1016/j.actbio.2017.07.014
1742-7061/