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Targeted Delivery of siRNA to Tumor Cells with Designed Ankyrin Repeat Protein Nanocomplexes

J. WINKLER [1,2], U. ZANGEMEISTER-WITTKE [2]

[1] Department of Medicinal Chemistry, University of Vienna, Althanstraße 14, 1090 Vienna, Austria
[2] Department of Pharmacology, University of Bern, Friedbühlstraße 49, 3010 Bern, Switzerland

E-mails: johannes.winkler@univie.ac.at (J. Winkler), uwe.zangemeister@pki.unibe.ch (U. Zangemeister-Wittke)

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Specific delivery to tumors and efficient cellular uptake of nucleic acids are major challenges for gene-targeted cancer therapies. Tumor-targeted delivery using antibody fragments or immunoliposomes have already been demonstrated to enhance cellular uptake of nucleic acids by receptor-mediated endocytosis. Here we report the first use of an epithelial cell adhesion molecule (EpCAM)-specific designed ankyrin repeat protein (DARPin) as a carrier for siRNA. Designed ankyrin repeat proteins are a novel class of non-immunoglobulin binding proteins relying on the modularity of ankyrins. Their short length, high stability, and the lack of intramolecular cysteines facilitate proper folding, result in high-yield expression in *E. coli*, and allow engineering procedures usually not well tolerated by antibodies. A DARPin binding to EpCAM was derived from a designed protein library using ribosome display.

For charge complexation of siRNA, the DARPin was fused to a truncated protamine peptide. In addition to the monomeric fusion protein, DARPin dimers for enhanced cell binding affinity were generated. Two different dimers, one in a head to tail arrangement, and the other in a head to head configuration using a self assembling leucine zipper, were engineered. All proteins expressed well in *E. coli* and were purified by affinity chromatography in high yields. To remove tightly bound *E. coli* nucleic acids, all fusion proteins were purified under denaturing conditions followed by on-column refolding.

The fusion proteins were capable of complexing up to five siRNA molecules per protamine, fully retaining the binding specificity for EpCAM. Upon EpCAM binding a 2'-*O*-methyl gapmer siRNA complementary to the bcl-2 mRNA was efficiently delivered only into tumor cells where it specifically downregulated the expression of anti-apoptotic bcl-2 both on the mRNA and the protein level. Inhibition of bcl-2 expression facilitated tumor cell apoptosis demonstrated by increased sensitivity to the anti-cancer agent doxorubicin. The DARPin dimer containing a leucin zipper showed higher binding avidity to EpCAM positive tumor cells, and siRNA delivery increased target downregulation and chemosensitization compared to the monomer. These results prove the possibility of using DARPins for siRNA delivery highlighting antigen affinity and loading capacity as important characteristics.

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