Development of Recombinant Proteins for Non Viral Gene Delivery Taking Advantage of Molecular Motor Proteins

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Abstract

The low efficiency of gene transfer is a recurrent problem in DNA vaccine development and gene therapy studies using non viral vectors such as plasmid DNA (pDNA). This is mainly due to the fact that during their traffic to the target cell´s nuclei, plasmid vectors must overcome a series of physical, enzymatic and diffusional barriers. The main objective of this work is the development of recombinant proteins specifically designed for pDNA delivery taking advantage of molecular motors, like dynein, for the transport of cargos from the periphery to the centrosome of mammalian cells. Here, a fusion protein containing an N-terminal dynein binding domain from human pericentrin, was fused to a DNA binding sequence at its C-terminus. Cloning was successfully performed in pET28a expression vector. Expression studies indicated that the fusion protein was correctly and best expressed in *E. coli* Rosetta strain. Purification studies are currently being performed by IMAC and ion-exchange chromatographies. After evaluation of the protein secondary structure using circular dichroism, *in vitro* assays will be performed to check the ability of the recombinant fusion protein to interact with both, plasmid DNA and the molecular motor dynein. By performing transfection studies using the recombinant proteins in combination with a model pDNA vector (pVAX1GFP), we expect to confirm the capacity of these constructs to facilitate intracellular pDNA trafficking through the cytosol and to the nucleus of the target mammalian cells. The development of recombinant shuttle proteins able to take advantage of strategies used by virus to infect mammalian cells may, in the near future, reduce the current advantage of viral vectors comparing to non viral vectors, providing new potent tools for gene therapy and DNA vaccination studies.