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Cell penetrating peptide POD mediates delivery of recombinant proteins to retina, cornea and skin

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ABSTRACT

Recently we described a novel cell penetrating peptide, peptide for ocular delivery (POD) that could deliver small molecules including fluorescent dyes into retinal cells. The objective of the current study was to examine whether biologically relevant macromolecules such as proteins, genetically fused with POD could also be delivered into retinal tissues *in vivo*. We generated a POD–GFP fusion protein and examined its cell and tissue penetrating properties. We found that endogenously expressed POD–GFP fusion protein localized to the nucleus, suggesting that POD acts as a nuclear localization signal. Adenovirus (Ad) vectors expressing POD–GFP fusion protein were constructed and the recombinant protein was purified from Ad-infected human embryonic retinoblasts (HER). Exogenously supplied POD–GFP fusion protein rapidly transduced A549 and HER cells and colocalized in part with markers of late endosomes, from which it could escape. Following subretinal delivery, POD–GFP localized to the retinal pigment epithelium and the photoreceptor cell bodies. When injected into the vitreous, POD–GFP localized to the ganglion cells and the inner nuclear layer of the retina as well as the lens capsule. Topical application of POD–GFP to ocular surfaces resulted in uptake by the corneal epithelium. POD–GFP also transduced non-ocular tissues, including the epidermis of the skin following topical application.

1. Introduction

According to public opinion polls, blindness is the second most feared condition amongst Americans after cancer. Almost 200 different loci and 130 different genes responsible for inherited retinal degeneration have thus far been identified, making retinal degeneration one of the most heterogeneous genetic disorders in humans (www.retnet.org). A large number of these genes are expressed exclusively in the photoreceptors or retinal neurons. Efficient delivery of therapeutic proteins into retinal cells is currently limited to the use of recombinant viruses for delivery of DNA expression cassettes – an approach that in some tissues can be deleterious (Donsante et al., 2007). Hence, relatively intensive and significantly protracted preclinical studies need to be performed prior to the use of gene delivery in humans. Given the large number of genes involved in retinal degeneration, preclinical testing of gene therapy approaches for every gene is prohibitive. Hence, one may envisage that progress towards the development of therapies for many retinal degenerative diseases may be accelerated by developing the theoretically less risky approach of protein delivery – administration of which can be readily terminated upon the initial observation of any adverse events. For example, although viral-mediated gene transfer of ciliary neurotrophic factor (CNTF) to animal models of retinal degeneration has been documented to be deleterious (Buch et al., 2006), phase I trials have been safely completed by use of encapsulated CNTF-producing cells that could be readily removed from the ocular compartment of retinitis pigmentosa patients (Sieving et al., 2006).

For some retinal degenerative diseases such as rhodopsin or peripherin/RDS-associated retinitis pigmentosa (RP), over-expression of therapeutic gene product may be deleterious, as has been observed with both viral gene transfer (Sarra et al., 2001) and in transgenic mice (Tan et al., 2001). Hence, therapies for RP patients could theoretically be safer by delivery of protein instead of DNA. In the context of retinal degeneration, which is typically a slow progressive disease, one may envisage the use of slow release formulations or depots in the intraocular environment for long-term delivery of therapeutic proteins as has been achieved previously in some clinical studies (Sieving et al., 2006). However, macromolecules do not readily cross the plasma membrane and do not penetrate neural tissues such as the retina.

Previously, several proteins exhibiting the unusual property of traversing lipid bilayers have been identified. These molecules contain protein transduction domains (PTDs) that can be chemically cross-linked to heterologous proteins, antibodies and enzymes and facilitate their transport across the plasma membrane

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(Anderson et al., 1993; Fawell et al., 1994). Protein transduction was first reported over a decade ago by Green and Frankel who independently demonstrated that the Human Immunodeficiency Virus (HIV) TAT protein was able to enter cells when added to the surrounding media (Frankel & Pabo, 1988; Green & Loewenstein, 1988). Subsequently, several other proteins with transducing capabilities have been identified, the most intensively studied of which are the Drosophila homeotic transcription factor ANTP (encoded by the antennapedia gene) and the Herpes Simplex Virus (HSV) VP22 (Elliott & O'Hare, 1997; Joliot, Pernelle, Deagostini-Bazin, & Prochiantz, 1991; Joliot, Triller, Volovitch, Pernelle, & Prochiantz, 1991). A variety of additional cell penetrating peptides have since been discovered (Vives, Schmidt, & Pelegrin, 2008). Apart from HIV TAT, there is very limited information available on the performance of PTDs in the retina (Barnett, Elangovan, Bullok, & Piwnica-Worms, 2006; Cashman, Morris, & Kumar-Singh, 2003; Dietz, Kilic, & Bahr, 2002; Schorderet et al., 2005).

Previously, we examined the potential use of HIV TAT (Cashman et al., 2003) and HSV VP22 (Cashman, Sadowski, Morris, Frederick, & Kumar-Singh, 2002) in delivering recombinant proteins to human embryonic retinoblasts in culture and to retinal tissues *in vivo*. We found that whereas both of these PTDs acted efficiently *in vitro*, their performance in the retina *in vivo* was limited. Hence, we recently developed and described a novel cell and tissue penetrating peptide referred to as POD – (peptide for ocular delivery; GGG[ARKKAAKA]$_4$) that could be used to efficiently deliver fluorescent dyes or other similarly small molecules into the retina *in vivo*.

The objective of the current study was to examine the potential of genetically fusing biologically relevant macromolecules such as whole proteins to POD in order to determine whether novel transduction properties are conferred upon the recombinant fusion protein in terms of delivery into the retinal photoreceptors and interneurons. For proof-of-principle, here we examine delivery of a POD–GFP fusion protein to retinal cells *in vitro, ex vivo* and *in vivo*. Our results indicate that POD-fusion proteins allow the penetration and dispersion of macromolecules into a variety of cells and tissues and hence may have significant therapeutic applications.

2. Results

2.1. De novo synthesized POD–GFP localizes to the nucleus

Transfection of human embryonic retinoblast (HER) cells (Fallaux et al., 1996) with pGFPHis, a plasmid expressing a His-tagged GFP, resulted in relatively diffuse cytoplasmic and nuclear localization of recombinant GFP (Fig. 1a). In contrast, transfection of HER cells with pPODGFPHis, a plasmid expressing a His-tagged POD–GFP fusion protein, resulted in relatively weak cytoplasmic and intense nuclear localization (Fig. 1b). Furthermore, POD–GFP appeared to concentrate in sub nuclear compartments (Fig. 1b, arrowheads). This pattern of localization associated with POD–GFP contrasts with that of exogenously added lissamine-conjugated POD peptide (L-POD), which we have previously shown to localize primarily to the cytoplasm in a punctate pattern – reminiscent of endocytosis (Johnson, Cashman, & Kumar-Singh, 2007). We conclude that endogenously expressed POD can act as a nuclear localization signal for POD-fusion proteins.

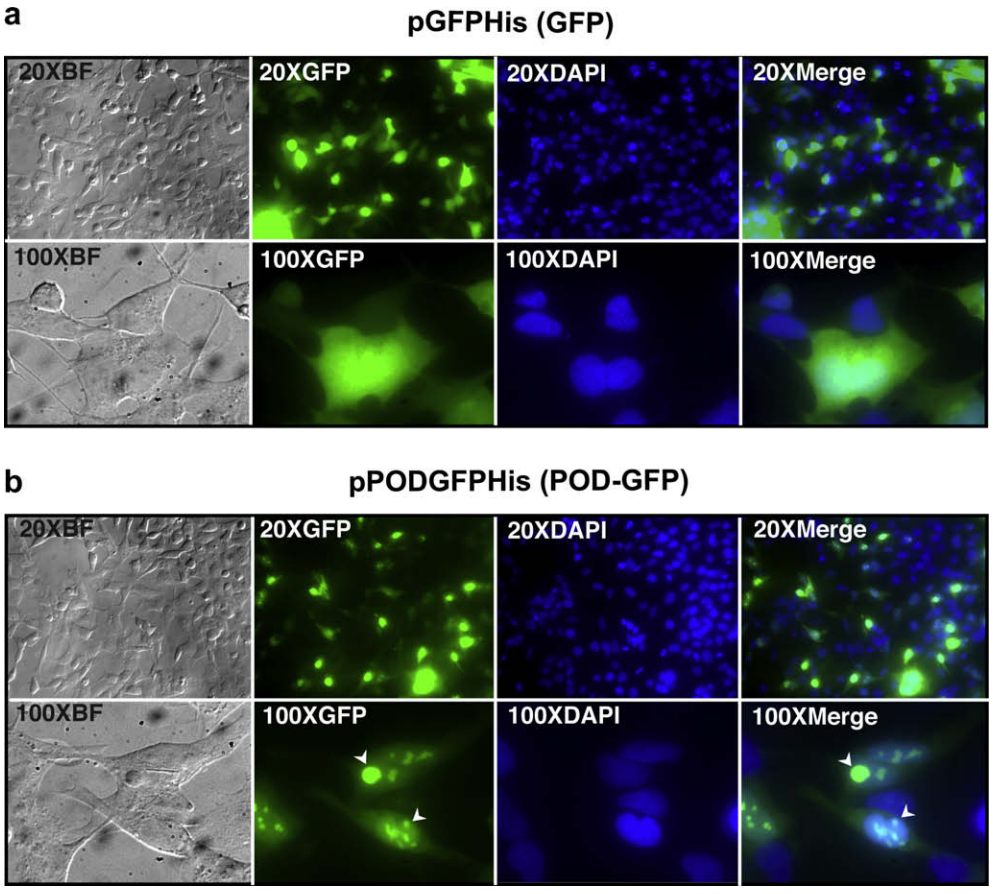

Fig. 1. De novo synthesized POD–GFP fusion protein localizes to the nucleus. HER cells were transfected with either pGFPHis (a) or pPODGFPHis (b), plasmids expressing His-tagged GFP or POD–GFP fusion proteins respectively. Whereas GFP localizes to the cytoplasm and nucleus (a), POD–GFP localizes primarily to the nucleus (b). Furthermore, POD–GFP appears to be concentrated in sub nuclear compartments (b, arrowheads). BF, brightfield. Color version of this figure appears online.