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Short communication

Protein therapy for Unverricht–Lundborg disease using cystatin B transduction by TAT-PTD Is it that simple?

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Abstract

In this work we analysed the characteristics of the cell-permeable peptide TAT-PTD fused to cystatin B (CSTB) to evaluate its potential for protein therapy of Unverricht–Lundborg (UL) epilepsy.

TAT-PTD–CSTB does not penetrate the cells despite initial evidence of time and concentration-dependent transduction. Therefore, it cannot be used as a form of replacement of the intracytoplasmic protein missing in UL. Importantly, we discuss precautions to avoid false-positive results when working with TAT-PTD for protein therapy of neurological diseases.

Keywords: Progressive myoclonus epilepsy; Unverrricht-Lundborg; Protein therapy; Blood–brain-barrier; Cell-permeable peptide; TAT-PTD

1. Introduction

Unverricht–Lundborg disease (ULD) is a chronic and debilitating autosomal recessive form of progressive myoclonus epilepsy, with onset between the ages of 6 and 15 years. Tonic–clonic and myoclonic seizures appear early in the clinical picture, but other types of seizures may present later. Stimulus-sensitive myoclonias are the presenting symptoms in 50% of patients. The disease progresses slowly with ataxia, action tremor and emotional lability. Widespread neurodegeneration is seen at autopsy (Koskiniemi et al., 1974). In almost all cases, ULD is caused by expansion of a dodecamer repeat in the promoter of the *EPM1* gene, which dramatically reduces the level of its ubiquitously expressed protein product named cys-

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tatin B (CSTB) (Lehesjoki et al., 1993; Pennacchio et al., 1996; Lafreniere et al., 1997). Replacement of CSTB in brain could lead to an improvement of symptoms or, at least arrest disease progression. However, the blood–brain-barrier (BBB) prevents the entry of viral vectors and most proteins, therefore patients need stereotatic surgery to deliver them. Since gene expression may fade and ULD is a life-long disease, patients would likely need repeated surgeries, which are fraught with risks and potential complications.

Recently, it was demonstrated that the TAT protein transduction domain (TAT-PTD), an 11 amino acid peptide derived from the human immunodeficiency virus (HIV-1), is able to transport proteins fused to it across the blood–brain-barrier and transduce them into neurons and other brain cells (Schwarze et al., 1999). Confirmation of this finding could cause a major impact in the development of new therapies for neurological diseases. Here, we test whether TAT-PTD can transduce CSTB into cultured cells. We chose to experiment with CSTB and ULD, because CSTB is a small cytoplasmic protein, which does not require organellar passaging or modifications. In addition, looking ahead to human studies, ULD patients express small amounts of CSTB and would not be expected to mount an immune response against the supplemented protein.

2. Methods

2.1. Plasmid construction

The *EPM1* coding region (I.M.A.G.E. clone 1383042, Research Genetics) was amplified using primers EPM1F: CGGTCTCGAGATGATGTGTG and EPM1R: CCGCAGCGAATTCGAAGCTT containing *Xho*I and *Eco*RI restriction sites, respectively. This PCR product was cloned into the pRSET vector

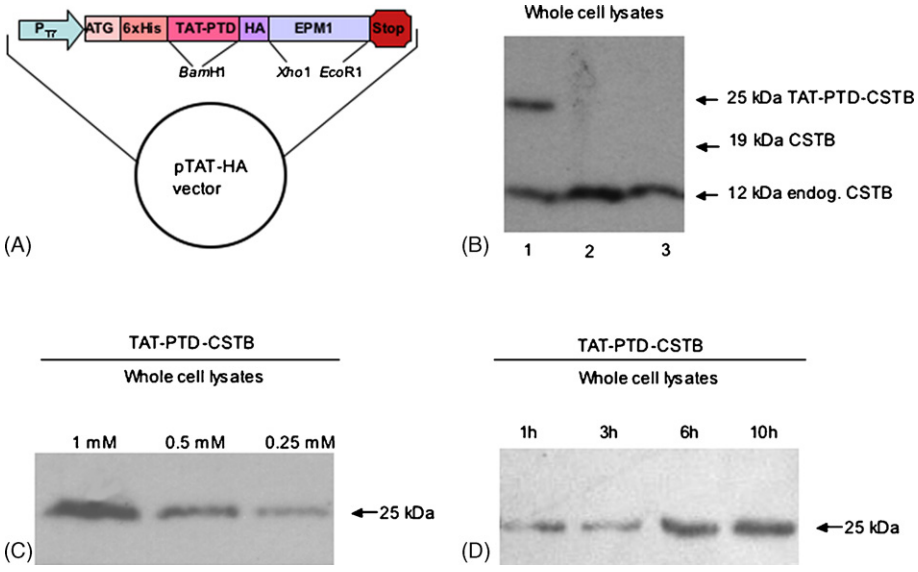

Fig. 1. (A) Plasmid assembly. Cystatin B cDNA *EPM1* inserted between *Xho*1 and *Eco*R1 restriction sites of the multiple cloning site of pRSET. Control protein generated by digestion of the above construct with *Bam*H1, thus removing TAT-PTD epitope. (B) Apparent transduction of CSTB is dependent on TAT-PTD (results in this and subsequent experiments were identical in COS-7 cells and lymphoblasts; only COS-7 data are shown). COS-7 cells incubated for 1 h with: (1) 1 mM TAT-PTD–CSTB, (2) 1 mM exogenous CSTB without TAT-PTD and (3) medium only. Western blot analysis demonstrates the presence of TAT-PTD–CSTB (25 kDa) but not exogenous CSTB (19 kDa) in whole cell lysates (the 12 kDa band is the endogenous CSTB). (C) Apparent concentration-dependent transduction of TAT-PTD–CSTB. COS-7 cells incubated with decreasing concentrations of TAT-PTD–CSTB (1, 0.5 or 0.25 mM) result in a progressively fainter 25 kDa band. (D) Apparent time-dependent transduction of TAT-PTD–CSTB. Intensity of the 25 kDa band increases with incubation time. Primary antibody used in all three panels was polyclonal anti-CSTB (ANAWA Zurich, Switzerland, 1:500). Secondary was anti-rabbit immunoglobulin against polyclonal primary antibody (Amersham Pharmacia, UK, 1:1000). The chemiluminescence reagents were from Perkin-Elmer (Life Sciences, MA, USA).