Biochemical and Biophysical Research Communications 394 (2010) 348–353

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Quantification of cellular uptake and *in vivo* tracking of transduction using real-time monitoring

Mi-Sook Lee [a,1,2], Eun-Hye Kwon [a,1], Hoon-Sung Choi [a], Seung-Hae Kwon [a], Chul-Hyun Lee [a], In-sop Shim [b], Sang-Kyou Lee [c], Song Her [a,*]

[a] Division of Bio-Imaging, Chuncheon Center, Korea Basic Science Institute, Chuncheon, Kangwon-Do 200-701, Republic of Korea
[b] Department of Medical Science, Graduate School of East-West Medical Science, Kyung Hee University, Seoul 130-701, Republic of Korea
[c] Department of Biotechnology, College of Engineering and Bioproducts Research Center, Yonsei University, Seoul 120-749, Republic of Korea

ARTICLE INFO

Article history:
Received 25 February 2010
Available online 4 March 2010

Keywords:
Protein transduction domain
Luciferase
Quantification
Cellular uptake
In vivo tracking

ABSTRACT

Protein transduction domains (PTDs) are short amino acid sequences that promote their own translocation across the cell plasma membrane and have been studied for possible use in drug delivery and gene therapy. However, no direct method to quantify transduction is available. Here, using a new luciferase-tagged human PTD, we show that cellular uptake levels can be determined in a reliable manner. Furthermore, we show that enhanced *in vivo* tracking by human PTD can be quantified in a mouse model. This is the first report on the direct quantification of PTD transduction *in vitro* and *in vivo*, which will be necessary for studying its possible therapeutic application in drug delivery and gene therapy.

1. Introduction

Efficient uptake of bioactive compounds into target cells is a key to achieving therapeutic efficacy. Protein transduction domains (PTDs) have been shown to efficiently deliver active macromolecules physiologically and therapeutically, such as peptides, proteins, nanoparticles, liposomes, phages, and viruses [1–6]. Previously, we reported a novel cell-permeable PTD from a human transcription factor (YARVRRRGPRR) that prevented allergic inflammation by intranasal delivery of the cytoplasmic domain of CTLA-4 using this hPTD [7]. However, evaluating the efficiencies of PTD-mediated delivery has been based on hPTD conjugated to fluorescence proteins or galactosidases. The indirect quantitative measurements of fluorescence intensity or galactosidase activity have lead to unreliable estimates of transduction efficiency due to artifacts in cell fixation and misinterpretation of the quantification of cell membrane-bound fluorescently-labeled PTD [8,9].

For real-time quantification of transduction, we introduced bioluminescence imaging (BLI) analysis which is a cutting-edge technology for molecular imaging of not only living cells, but also small animals, providing high sensitivity and consistent reproducibility along with the advantage of non-invasive quantification [10]. BLI is based on the bioluminescent measurement of luciferase activity. In living cells, the luciferase catalyzes luciferin, which emits light in the presence of intracellular ATP (Fig. 1A). Using the fact that the reaction catalyzed by luciferase takes place when cells take up exogenous luciferase, we designed a luciferase-tagged hPTD to monitor real-time intracellular transduction of hPTD. In this study, using luciferase-tagged hPTD, we accurately quantified enhanced cellular uptake and *in vivo* tracking by hPTD.

2. Materials and methods

2.1. Construction of the hPTD-Luc expression vector

The firefly luciferase gene from plasmid pGL3 (Invitrogen Corporation, Carlsbad, CA, USA) was amplified using forward (5′-CCGGAATTCATGGAAGACGCCAAAAACAT-3′) and reverse (5′-CCCAAGCTTTTACACGGCGATCTTTCCGC-3′) primers with incorporated EcoRI and HindIII restriction sites, respectively. The PCR amplified fragments then replaced EGFP in pRSET Hph1 [7] to produce pRSET-hPTD-Luc. To create pRSET Luc, the control, pRSET-hPTD-Luc was double-digested with NheI/EcoRI to delete hPTD, followed by self-ligation. All of the constructs were verified by restriction mapping, and the junction of the gene within the constructs was confirmed by sequencing at the DNA sequencing facility of Macrogen, Inc. (Seoul, Korea).

* Corresponding author. Fax: +82 33 255 7273.
E-mail address: swher@kbsi.re.kr (S. Her).
[1] These authors contributed equally to this work.
[2] Current address: Department of Medical Science, Graduate School of East-West Medical Science, Kyung Hee University, Seoul 130-701, Republic of Korea.

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.03.009

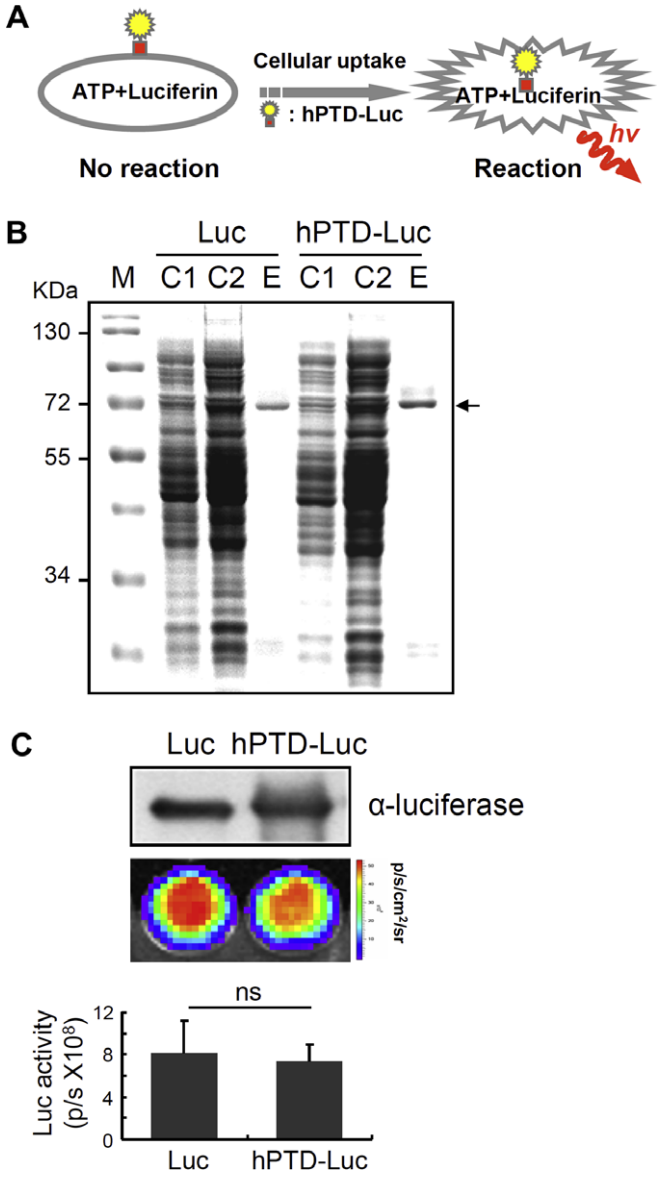

Fig. 1. hPTD-Luc fusion protein. (A) Diagram of luminescence monitoring by cellular uptake of hPTD-Luc. (B) Image of Coomassie blue staining from SDS–PAGE analysis. Expression of Luc and hPTD-Luc protein in *E. coli* BL21(DE3) before (C1) induction by 1 mM IPTG and eluted protein (E) from post-IPTG treated cell lysates (C2). The arrow indicates the position of the 61-kDa luciferase. (C) Analysis of purified Luc and hPTD-Luc by Western blot (top) and luciferase activity (middle). The luciferase activity was quantified in p/s by the IVIS-200 (bottom). Data represent means ± SEM from three independent measurements. ns, non-significant.

2.2. Expression and purification of proteins

Protein expression and purification were performed as described previously [7]. Expression levels in cell lysates and purified proteins were characterized by SDS–PAGE and Western blotting, as described previously [11].

2.3. Luciferase assay

Luc and hPTD-Luc purified protein was assayed for luciferase activity using the Luciferase Assay System (Promega, Madison, WI, USA) according to the manufacturer's protocol, as described previously [12].

2.4. In vitro transduction

HeLa cells were cultured to 70% confluence in black-walled 96-well microtiter plates. Just before transduction, the culture medium was removed and replaced with 0.1 mL fresh medium containing Luc or hPTD-Luc. D-Luciferin (potassium salt; Promega) was added to a final concentration of 150 μg/mL, and then photon counts were acquired for 1 min.

2.5. In vivo transduction and BLI

For *in vivo* BLI measurement, each ICR mouse was injected intraperitoneally (ip) once with the same specific luciferase activity of 2 mg/kg hPTD-Luc or Luc. Then, the mouse was given an injection of D-luciferin at a dose of 30 mg/kg every 2 h. General anesthesia was given, and BLI analysis was performed as described previously [13]. The BLI intensity in the regions of interest was expressed as a total flux in units of photons per second (p/s). An *ex vivo* optical imaging assay was performed on dissected tissues. The animals were sacrificed 15 min after injection of D-luciferin,

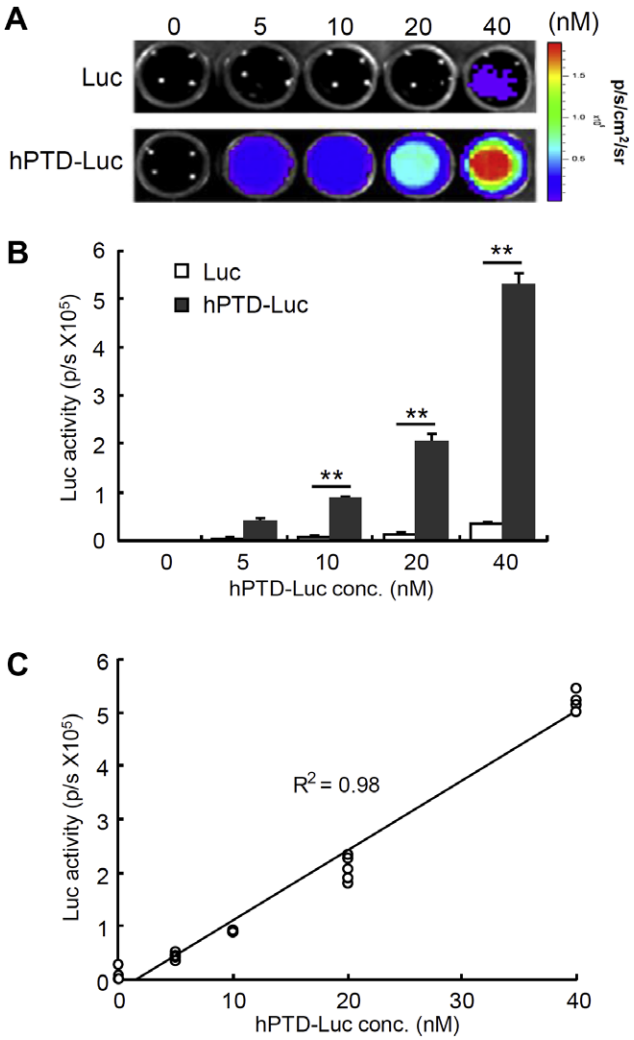

Fig. 2. Cellular uptake of hPTD-Luc in HeLa cells. (A) Representative image and (B) quantification of luciferase activity in the presence of Luc or hPTD-Luc with the indicated dose for 5 min. Data represent means ± SEM from each assay, performed in triplicate with three independent experiments. **Significantly different from Luc-treated cells ($p < 0.01$). (C) Correlation between BLI intensity (*Y*-axis) and hPTD-Luc dose (*X*-axis). The Spearman's rank correlation coefficient (R^2) is indicated.