Amino Acids (2014) 46:2595–2603
DOI 10.1007/s00726-014-1798-8

ORIGINAL ARTICLE

Enhanced cellular uptake of a TAT-conjugated peptide inhibitor targeting the polo-box domain of polo-like kinase 1

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Received: 21 April 2014 / Accepted: 25 June 2014 / Published online: 24 August 2014

Abstract In the last decade, drug delivery systems using biologically active molecules for cellular uptake of therapeutic targets have been studied for application and testing in clinical trials. For instance, the transactivator of transcription (TAT) peptide, or cell-penetrating peptide, was shown to deliver a variety of cargoes, including proteins, peptides, and nucleic acids. Polo-like kinase 1 (Plk1) plays key roles in the regulation of cell cycle events (e.g., mitotic progression). Plk1 was also shown to be activated and highly expressed in proliferating cells such as tumor cells. Amongst these phosphopeptides, Pro-Leu-His-Ser-p-Thr (PLHSpT), which is the minimal sequence for polo-box domain (PBD) binding, was shown to have an inhibitory effect and to induce apoptotic cell death. However, the phosphopeptide showed low cell membrane penetration. Thus, in our study, we synthesized Plk1 inhibitor TAT-PLHSpT to improve agent internalization into cells. TAT-PLHSpT was shown to internalize into the nucleus. The conjugation of TAT with PLHSpT inhibited cancer cell growth and survival. Moreover, it showed an increase in cellular uptake and inhibition of Plk1 kinase activity. Further studies are needed for biological evaluation of the new peptide in tumor-bearing animal models (in vivo). Our results prove that TAT-PLHSpT is a good candidate for specific PBD binding of Plk1 as a therapeutic agent for humans.

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Keywords Polo-like kinase 1 · Polo-box domain · PLHSpT · Transactivator of transcription · Cancer cell · Therapeutic agent

Introduction

Cancer is currently the key reason for an increased death rate worldwide. Therefore, many therapeutic agents have been developed with the aim to cure cancer-related diseases, albeit with limitations such as toxicity, cell penetration, pharmacokinetics, and stability of biologically active molecules. Most drug development research has focused on efficient drug delivery systems. Drug delivery systems have become important for cellular uptake of therapeutic targets and depend on a biologically active molecule's size, charge, or synthesis. Approaches for enhancing drug delivery for rapid uptake by cells have been studied during the last decade for application and testing in clinical trials. Cell-penetrating peptides have been mostly known for cellular uptake and can be internalized in most cell types (Richard et al. 2003). They allow cellular delivery and are generally applied as conjugated forms, such as hard cellular penetrable biomolecules, antigenic peptides, nucleic acids, antisense oligonucleotides, proteins, nanoparticles, and liposomes (Schwarze et al. 2000; Shibagaki and Udey 2002; Pooga et al. 1998; Fawell et al. 1994; Nagahara et al. 1998; Lewin et al. 2000; Torchilin et al. 2001). The cationic transactivator of transcription (TAT) peptide is a cell-penetrating peptide consisting of arginine-rich peptides. It has been shown to successfully deliver a large variety of cargoes such as proteins, peptides, and nucleic acids into primary cells, as well as into most tissues in preclinical models, and is currently being applied in human trials (Gump and Dowdy 2007). Moreover, most of the TAT-conjugated molecules have been accompanied by

the expected biological activity (Vives 2003). Despite a high number of applications using TAT-mediated peptides, the precise mechanism of its entry into cells is still unclear. The cationic charges of TAT peptides certainly play a key role in the uptake process and allow for their functional internalization into cells or tissues (Vives et al. 1997; Wender et al. 2000; Ferrari et al. 2003). According to some publications, TAT peptide-conjugated particles are efficiently translocated into the cell nucleus (de la Fuente and Berry 2005; Josephson et al. 1999). Therefore, the TAT peptide has been widely used to improve cellular delivery with bioactive molecules that are poorly taken up into cells.

The polo-like kinase (Plk) family, which includes four human serine/threonine protein kinases (Plk1, Plk2, Plk3, and Plk4), was shown to be an important family of proteins in cell cycle regulation, cell division, and even proliferation (Liu et al. 2011; Barr et al. 2004; Dai 2005). For instance, Plk1 is known to play key roles in the regulation of mitotic progression, spindle formation, chromosome segregation, and cytokinesis (Lansing et al. 2007; Barr et al. 2004). In addition, Plk1 has been shown to be highly expressed and to play the main role in proliferating cells, especially in a variety of tumor cells (Strebhardt and Ullrich 2006). Therefore, many studies developed anticancer therapeutic agents by inhibiting the Plk1 kinase domain (Gumireddy et al. 2005; Lenart et al. 2007; Strebhardt 2010). Plk1 consists of two domains, the N-terminal kinase domain and the C-terminal polo-box domain (PBD), which is known as a phosphopeptide-binding domain. PBD inhibitors have been developed using small molecules or peptides (Reindl et al. 2008; Watanabe et al. 2009; Li et al. 2009). PBD binds Plk1 target proteins and its specific synthetic phosphopeptide sequence, PMQSpTPL, which has been shown to have a high affinity for Plk1 (Elia et al. 2003a). Recently, artificial phosphopeptides, which specifically interact with the PBD, have been optimized to target PBD (Cheng et al. 2003; Elia et al. 2003b; Yun et al. 2009). One of these phosphopeptides, Pro-Leu-His-Ser-p-Thr (PLHSpT), which is the minimal sequence for PBD binding, was shown to be inhibited by Plk1 PBD binding to induce apoptotic cell death (Yun et al. 2009). However, the minimal phosphopeptide has a limitation of poor cell membrane penetration, and owing to this disadvantage, it could not be used for human clinical studies. Therefore, this phosphopeptide needs to be modified to enable penetration into cancer cells and be used as a successful therapeutic agent.

In this study, we deliberated that drug delivery systems might benefit from this phosphopeptide. Thus, we hypothesized that the attachment of the TAT peptide might result in high levels of cell internalization and a high affinity to PBD-binding sites in cancer cells (Fig. 1). We designed and synthesized Plk1 inhibitor TAT-PLHSpT for improving internalization into cancer cells and characterized its inhibition of cancer cell proliferation. Our results suggest that TAT-PLHSpT might be a good candidate for specific PBD binding of Plk1 as a therapeutic agent in humans.

Experimental section

Chemicals

All chemicals were used without further purification. Diisopropylethylamine (DIEA), fluorescein 5(6)-isothiocyanate (FITC), triisopropylsilane (TIPS), and trifluoroacetic acid

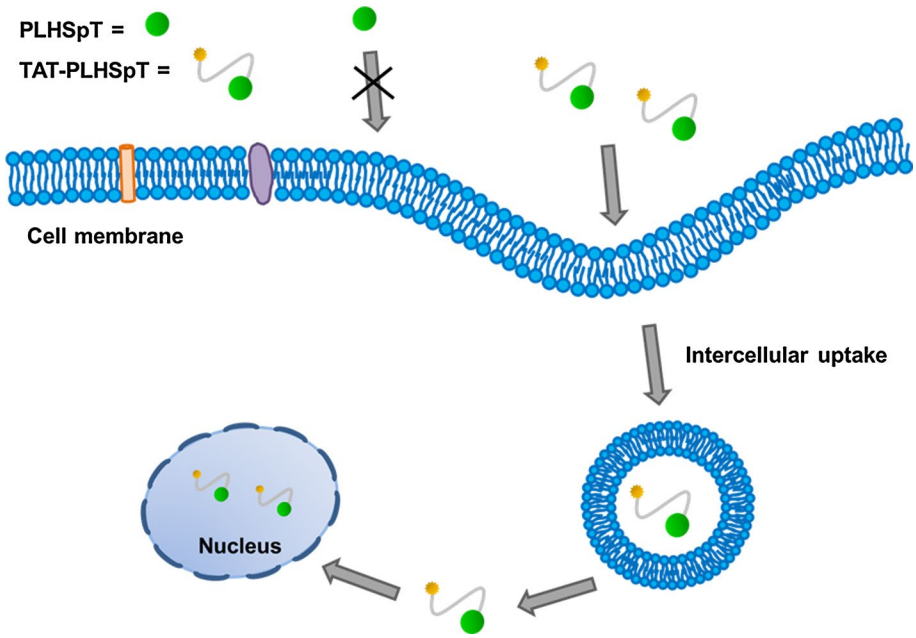

Fig. 1 Illustration of TAT-PLHSpT cellular uptake

(TFA) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). 9-Fluorenylmethyloxycarbonyl (Fmoc)-protected amino acid derivatives, Rink amide AM resin, *O*-benzotriazole-*N*,*N*,*N*,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU), and hydroxybenzotriazole (HOBt) were purchased from BeadTech Inc. (Seoul, Korea). HPLC was performed on a Young Lin HPLC system (C18, GRACE, Albany, OR, USA), along with the following gradient eluents: H_2O/0.05 % TFA and acetonitrile/0.05 % TFA; 10–90 % acetonitrile for 30 min at a flow rate of 2 mL/min. UV detection was carried out under a wavelength of 230 nm. Mass spectra were obtained by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (KBSI, Ochang Center, Korea).

Peptide synthesis

All peptides used in this study were chemically synthesized by solid-phase peptide synthesis. As shown in Fig. 2, Rink amide AM resin (100–200 mesh, 0.70 mmol/g) was swollen in dimethylformamide (DMF) for 50 min and treated twice with a 20 % (v/v) piperidine in DMF for 10 min. The resin was then washed five times with DMF and coupled with a DMF solution of Fmoc-protected amino acid (5 equiv.), HBTU (5 equiv.), HOBt (5 equiv.), and DIEA (10 equiv.) for 50 min. The resin was further washed five times with DMF. This procedure was repeated until the desired peptide sequence was obtained. The peptide was cleaved from the resin by treatment with TFA:TIPS:H_2O (90:5:5 v/v/v) for 2 h, and precipitated with cold diethyl ether. The resulting white powders were purified by preparative reverse phase high performance liquid chromatography (RP-HPLC) over a C_{18} column, with a liner gradient over 30 min of acetonitrile/0.05 % TFA from 10 to 90 %, at a flow rate of 2 mL/min. The final product was kept at −20 °C until further use.

In vitro assay: cellular uptake of TAT-PLHSpT

Overexpression of Plk1 has been observed in various human tumors (Li et al. 2009; Strebhardt and Ullrich 2006). Therefore, in this study, the following human cancer cell lines were used: HeLa (human epithelial carcinoma cell line), KB (human epidermoid carcinoma), and HT-29 (human colon adenocarcinoma), all of which were purchased from American type culture collection (ATCC, Manassas, VA, USA). HeLa and KB cells were grown in Dulbecco's modified eagle medium (DMEM; Gibco BRL, Grand Island, NY, USA), and HT-29 cells were grown in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco) supplemented with 10 % (v/v) fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA), 100 U/mL of penicillin, and 100 μg/mL of streptomycin (Gibco) in 5 % CO_2 humidified air at 37 °C.

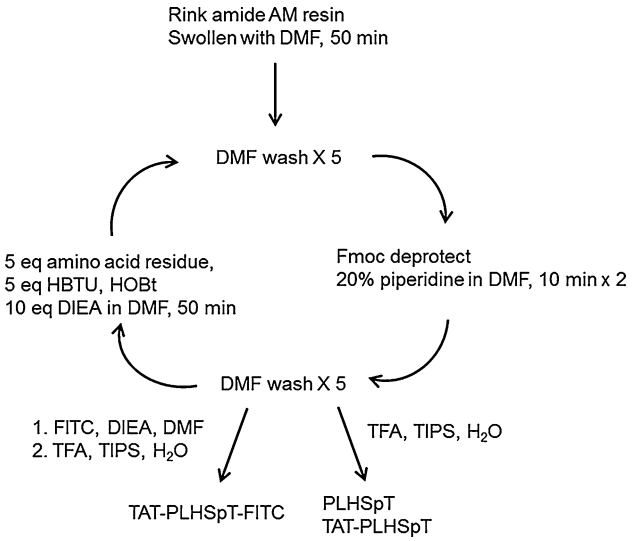

Fig. 2 Schematic of TAT peptide and phosphopeptide derivative via solid-phase synthesis

The uptake test of TAT-PLHSpT by cancer cell lines was performed by standard techniques using FITC-conjugated methods. Plated at 2×10^4 cells/well (HeLa, KB, and HT-29 cells), these cancer cell lines were cultured in 24-well polystyrene plates with plastic coverslips (Nunc™ Thermanox™ Coverslips, Thermo Fisher Scientific Inc., Waltham, MA, USA) for 24 h. Then, cultured media were changed to FBS-free DMEM media, including 5 μM of FITC-conjugated TAT-PLHSpT, and incubated for 3 h. Coverslips were rinsed three times with PBS, following which they were mounted with mount solution (Vector Shield, Vector Labs, Burlingame, CA, USA) using 1.5 μg/mL of 4′,6-diamidino-2-phenylindole (DAPI). The results were imaged by fluorescence microscopy (Olympus IX81, Olympus Inc., Japan). Images were processed using image analysis program software (MetaMorph Molecular Devices Inc., Sunnyvale, CA, USA).

Cell viability study

The XTT assay was used to assess the toxicity of the peptides. HeLa, U87MG, and HT-29 cells were seeded in 96-well microplates at 1×10^3 cells/well, in culture media containing 10 % FBS, and cultured for 24 h. Subsequently, the medium was replaced by DMEM containing 1 % FBS and different concentrations (0.1, 0.2, 0.5, 1, 2, 5, 10, and 50 μM) of peptides (TAT, PLHSpT, and TAT-PLHSpT) and incubated for 24 h at 37 °C. Survival ability of cancer cell lines was observed using the cell proliferation kit II (XTT) (Roche Applied Science, Penzberg, Germany). This experiment was repeated three times in duplicate.

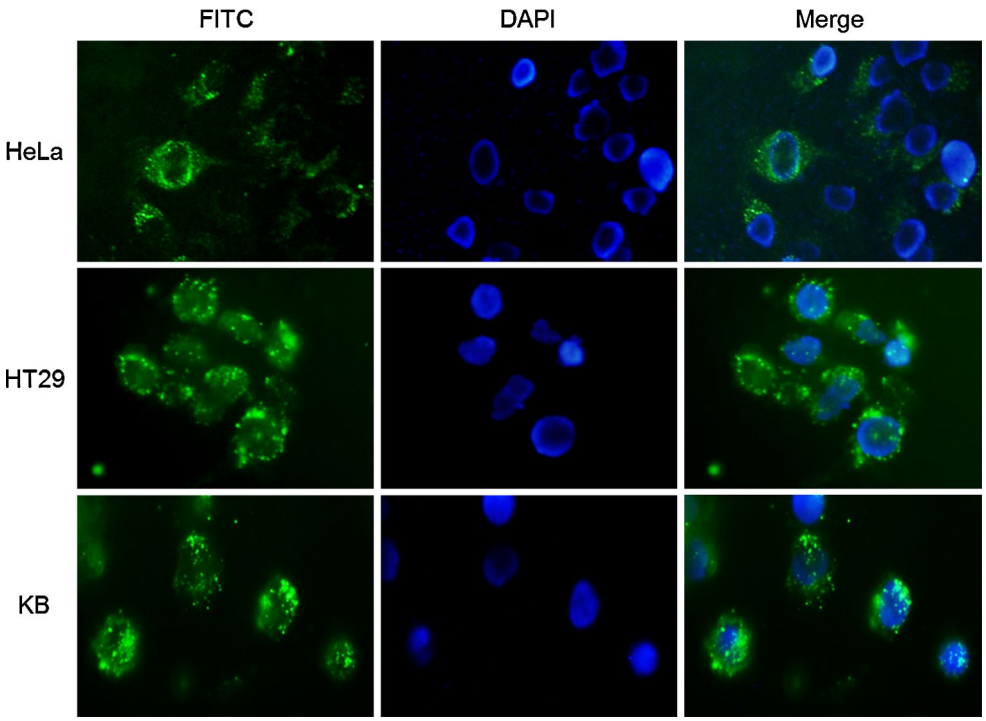

Fig. 3 Cellular uptake of TAT-PLHSpT by HeLa, HT29, and KB cancer cell lines. The cells were incubated with FITC-conjugated TAT-PLHSpT (*green*), and the cancer cell nuclei were stained with DAPI (*blue*). Cell imaging was performed using a fluorescence microscope (color figure online)

Plk1 kinase inhibition study

In order to analyze the Plk1 kinase inhibition effect by fusion peptide in vitro, we followed the CycLex® Polo-like kinase Assay/Inhibitor Screening Kit (MBL Inc., Tokyo, Japan) manual. Cancer cells (2×10^5 cells/well) were seeded in 6-well plates and cultured for 24 h. Then, the cells were incubated in media with peptides (5 μM of TAT, PLHSpT, and TAT-PLHSpT) for 24 h and 48 h. Next, the absorbance of Plk1 kinase activity in each well was measured with a spectrophotometric plate reader at 450 nm.

Cell cycle analysis

The effect of cell division inhibition in HeLa, KB, and HT-29 cells by TAT-PLHSpT was studied by double staining with antibodies. Cancer cells (2×10^4 cells/well) were cultured in 24-well polystyrene plates with plastic coverslips for 24 h and incubated in media with 10 μM of TAT, PLHSpT, and TAT-PLHSpT, for 24 h. The coverslips were then washed with PBS three times and fixed with 4 % paraformaldehyde. After treatment with a 1:100 dilution of Plk1 antibody (Abcam, Cambridge, MA, USA), coverslips were incubated for 1 h at room temperature. Then, coverslips were washed with PBS and incubated in PBS with 1:200 dilution of FITC-conjugated secondary antibody (Santa Cruz Biotechnology, Inc., CA, USA; 1:200 dilutions) for 3 h at room temperature. For DAPI double staining of chromosomes, VECTASHIELD Mounting Medium with DAPI (Vector Labs; 1:100 dilutions) was used. All images were procured on a fluorescence microscope (magnification ×600; Olympus IX81). All studies were performed in triplicate.

Acridine orange staining

Cells (2×10^4 cells/well) were cultured in 24-well polystyrene plates containing plastic coverslips. Each well was treated with 10 μM of peptides (TAT, PLHSpT, and TAT-PLHSpT) and incubated for 24 h. Then, the coverslips were fixed with 4 % PFA for 30 min, after which they were stained with 100 μg/mL of acridine orange/ethidium bromide solution (AO/EtBr; Sigma Aldrich) for 15 min at room temperature. The coverslips were washed five times with PBS. The results were imaged on a fluorescence microscope (magnification ×200; Olympus IX81) using a blue filter. Images were processed using Image analysis program software (MetaMorph Molecular Devices Inc.).

Western blot analysis

The apoptotic effect caused by peptides was repeated using apoptosis-related antibodies [caspase-3, poly (ADP-ribose)

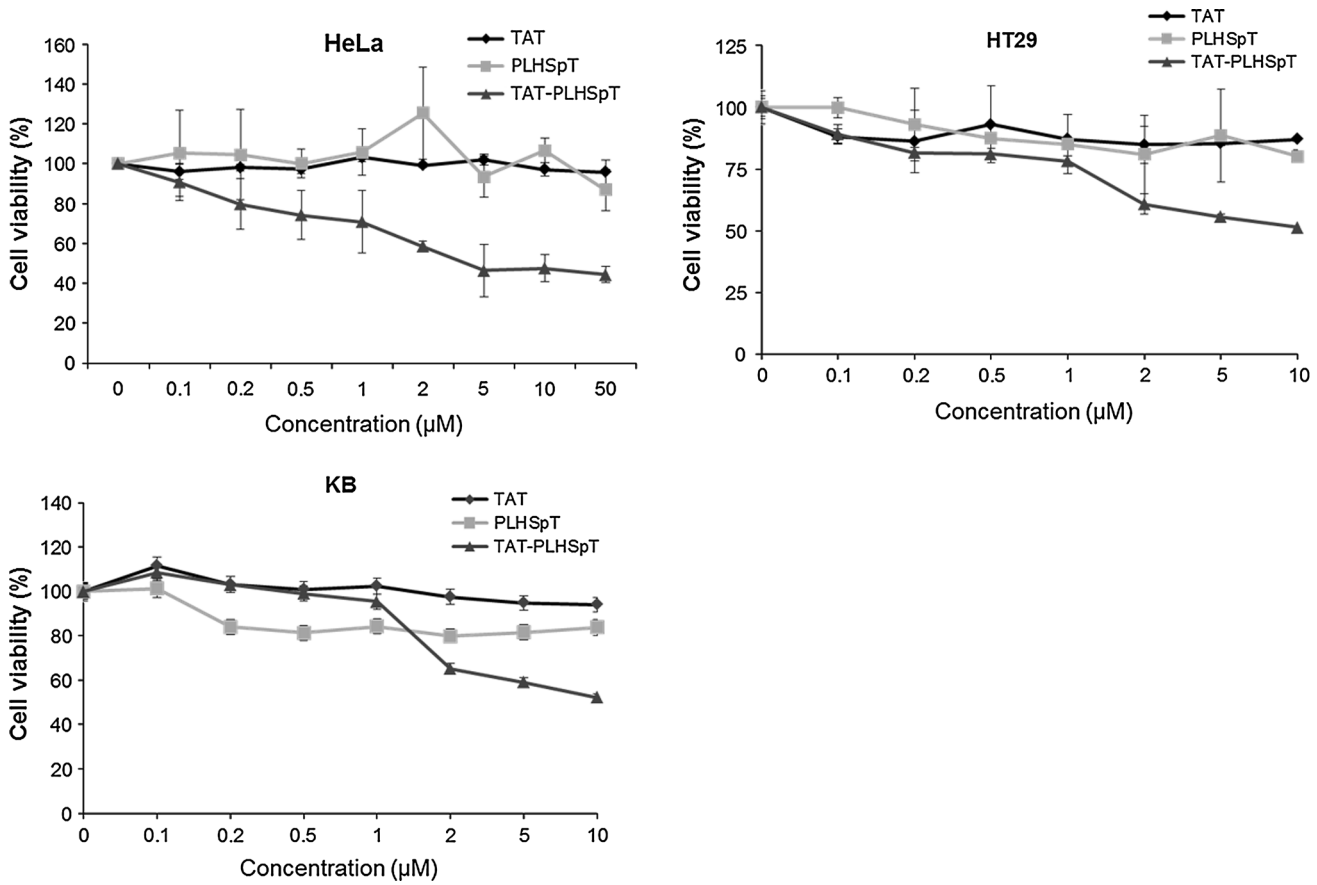

Fig. 4 Cell viability with TAT-PLHSpT treatment of HeLa, HT29, and KB cells. Cell proliferation rate (XTT assay) after 24 h of stimulation with TAT-PLHSpT of various concentrations (0, 0.1, 0.2, 0.5, 1, 2, 5, 10, and 50 μM)

polymerase (PARP), and cleaved PARP]. Total protein was extracted using PRO-PREP protein extraction solution (iNtRON Biotech. Inc., Daejeon, Korea) from the cells that were incubated for 24 and 48 h with 10 μM of peptides. In turn, total protein concentration was measured with the BCA protein assay kit (Merck Millipore, Darmstadt, Germany). Protein concentration of 20 μg/lane was used. The protein extracts were boiled for 5 min, loaded, separated by SDS–PAGE, and transferred to PVDF membranes. The membranes were then probed with appropriate antibodies (anti-PARP, and anti-caspase-3, Invitrogen; anti-beta actin, BD Bioscience). The signal was developed using an enhanced chemiluminescence detection system (Amersham-Pharmacia Biotech, Piscataway, NJ, USA).

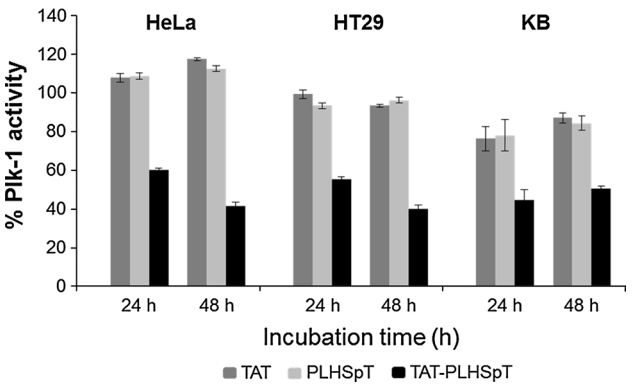

Fig. 5 Plk1 kinase activity with TAT-PLHSpT (5 μM) in the HeLa, HT29, and KB cells after 24 and 48 h incubations

Results and discussion

We developed a new specific Plk1 PBD inhibitor, which is cell permeable and can be used for cancer drug development. In general, while using biologically active molecules for high-efficiency intracellular drug delivery, most current techniques utilize one of two approaches: internalizing molecules by endocytosis or receptor-mediated endocytosis/phagocytosis. In our previous studies, we showed an efficient drug delivery system by receptor-mediated endocytosis with receptor-inhibiting peptide-conjugated PLHSpT, which is RGDyK-S–S-CPLHSpT

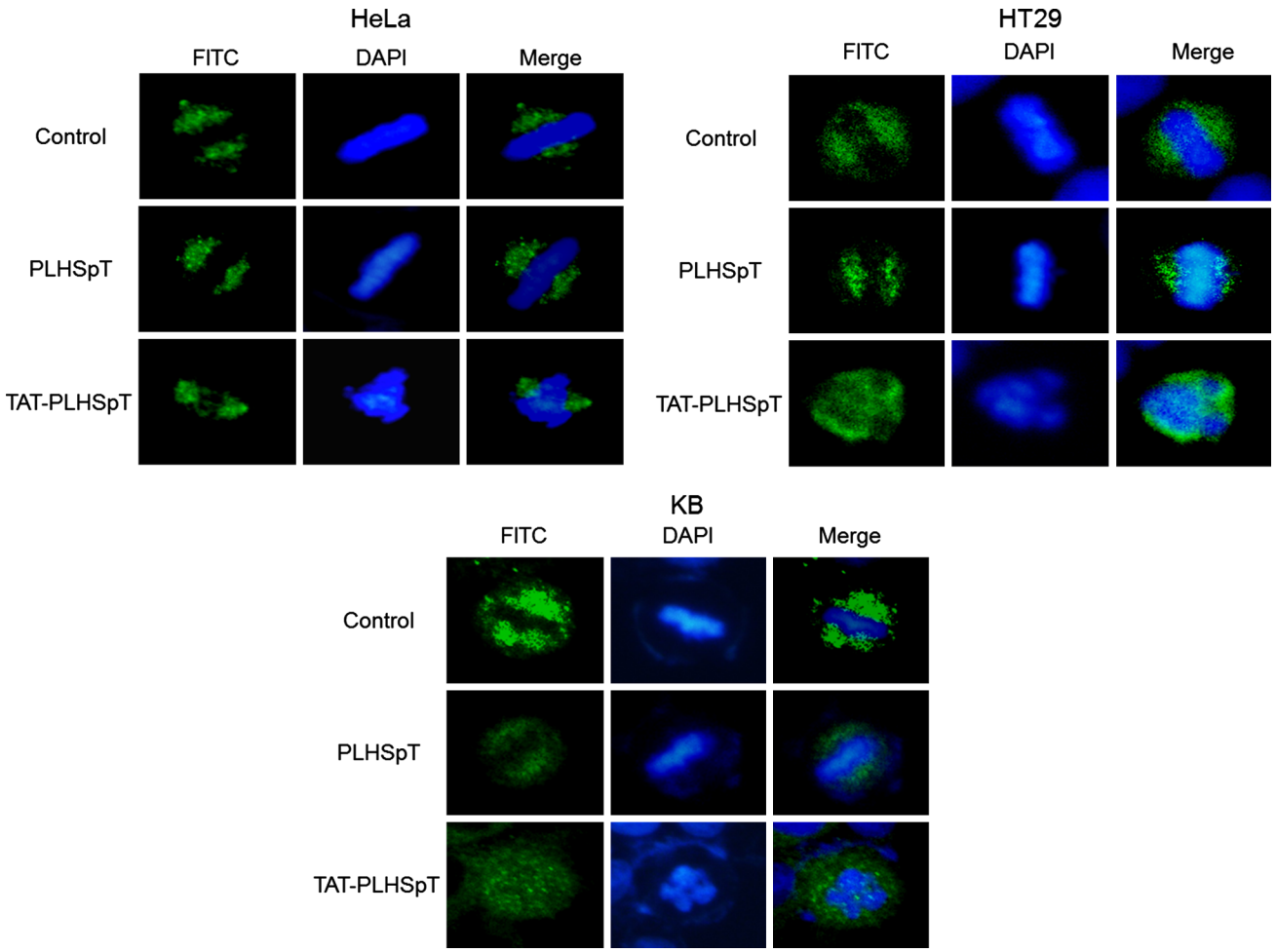

Fig. 6 Cell cycle analysis of TAT-PLHSpT (10 μM) in HeLa, HT29, and KB cells after 24 h. Centrosomal and spindle abnormalities were found in cancer cells with the peptide (magnification ×600)

(Kim et al. 2012). The conjugated peptide's cellular uptake was enhanced, and it showed anticancer activities not only in vitro (cancer cell lines) but also in vivo (tumor-bearing xenograft mice). Despite a high number of applications for the TAT peptide, the mechanism of internalization of TAT and its conjugated molecules is yet to be understood. In present studies, we used cell-penetrating peptides for cellular uptake along with a poorly internalized peptide. A TAT peptide with a sequence of YARVRRRGPRR was prepared by the solid-phase synthesis method on Rink amide resin (Fig. 2). The specific peptide with high affinity to PBD-binding sites, PLHSpT, could easily and sequentially conjugate to TAT-PLHSpT, the ideal cell-penetrable peptide. Solid-phase synthesis has advantages for peptide synthesis. In particular, it is needed in mild condition peptide synthesis. It allows in situ synthesis without any inconvenient intermediate purification procedure, and it can easily condense each amino acid to a desired peptide sequence.

We also synthesized a new inhibitor, TAT-PLHSpT, purified it by HPLC, and then identified it by MALDI-TOF spectrometry. PLHSpT, TAT, and PLHSpT-TAT, were purified by HPLC (Rt = 7.12, 6.56, and 10.63 min, respectively) and identified by MALDI-TOF (m/z = 633.29, 1442.0, and 2057.2, respectively).

For the evaluation of the peptide's penetration into cancer cells, we performed cellular uptake and cell proliferation studies by TAT-PLHSpT treatment. The cellular uptake ability of PLHSpT into cancer cells (HeLa, HT-29, and KB) was significantly increased by TAT conjugation. High cellular uptake of the peptide was evident by fluorescence microscopy in the three types of cancer cells. Moreover, the in vitro assay revealed that the cellular uptake of TAT-PLHSpT was mainly in the nuclear region of all three cancer cell lines studied (Fig. 3).

To test the inhibition effect of tumor cell growth by the peptides (TAT, PLHSpT, and TAT-PLHSpT), we performed a 24-h stimulation with DMEM containing 1 %

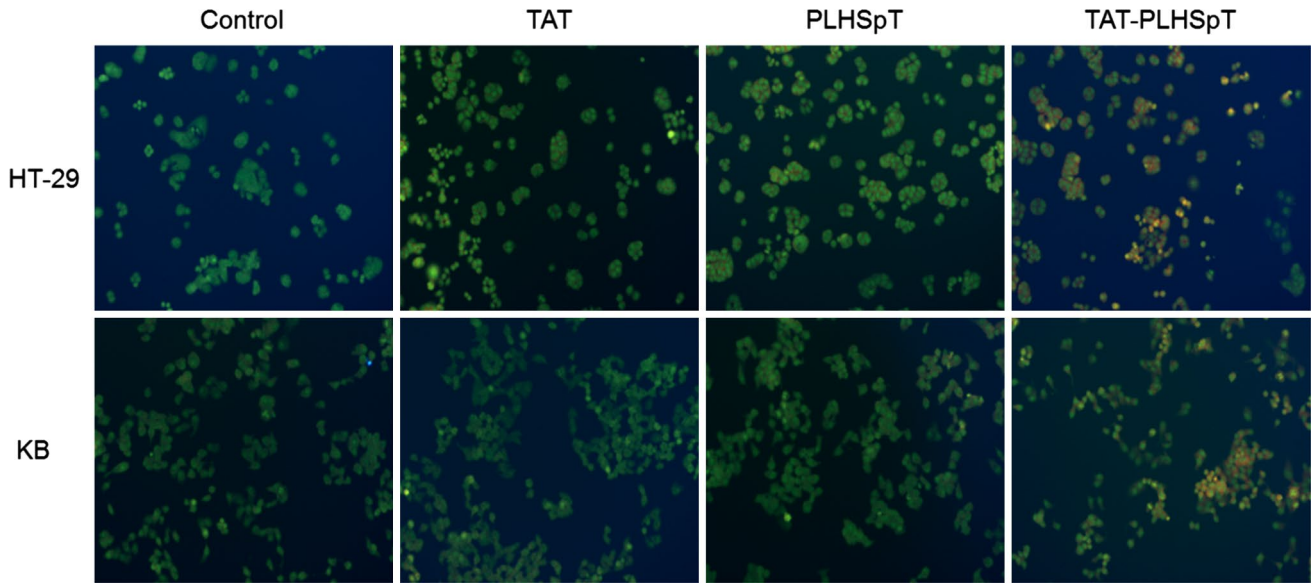

Fig. 7 Detection of apoptosis. HeLa, HT29, and U87MG cells were stained with acridine orange, and all images were obtained with a fluorescence microscope (magnification ×200)

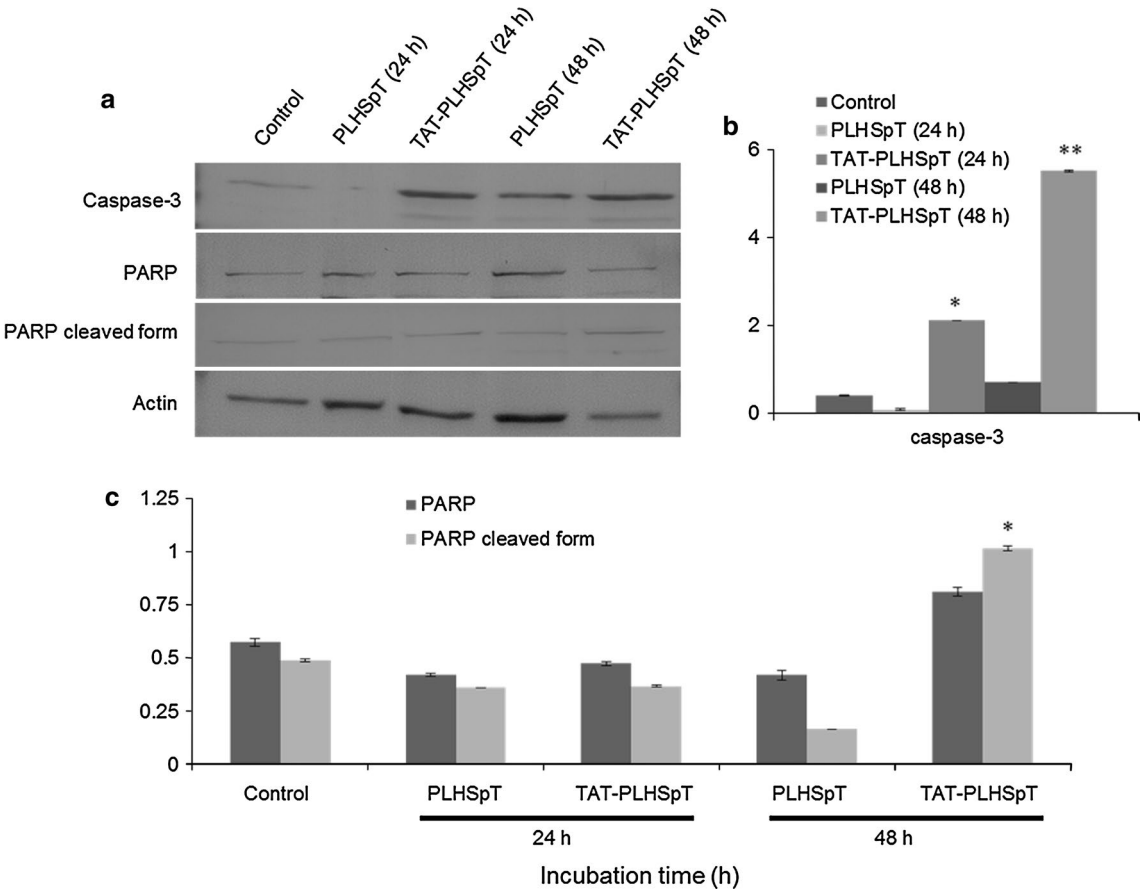

Fig. 8 Apoptosis effect of TAT-PLHSpT. **a** Western blot analysis of apoptotic cancer cells after incubation with TAT-PLHSpT for 24 and 48 h. **b** and **c** apoptosis was assessed by caspase-3 activation and PARP cleavage after incubation with TAT-PLHSpT for 24 and 48 h (*$P < 0.05$ and **$P < 0.01$)

FBS with various concentrations (0.1, 0.2, 0.5, 1, 2, 5, 10 and 50 μM) of peptides (Fig. 4). We found that the survival rate of cancer cells was significantly decreased in all cancer cell lines (HeLa, HT-29, and KB) in a dose-dependent manner with TAT-PLHSpT. The reduction of survival rate of cancer cells was prominent after treatment with concentrations of more than 1 μM of TAT-PLHSpT, although the effect of growth inhibition by TAT and PLHSpT was not apparent. In addition, during the survival inhibition effect test, we identified that the inhibitory concentration 50 % was attained after treatment with approximately 7.5 μM concentration. As a positive control, TAT-PLHSpT was compared with the PEGylated PLHSpT derivative (**9**) from a previous study (Liu et al. 2011). In that report, the PEGylated PLHSpT derivative was prepared by incorporation of polyethylene glycol into a PLHSpT for enhancing its membrane transport. Direction incubation of **9** with cultured HeLa cells was performed, and **9** was shown to effectively inhibit cell proliferation in a dose-dependent manner, with an IC_{50} of 380 μM. This was attributed to be low cell permeability of the compound. TAT-PLHSpT also showed a similar pattern in the cell morphology compared to **9**. However, direct incubation of TAT-PLHSpT with cultured HeLa cells had an IC_{50} value of 7.5 μM, indicating that TAT-PLHSpT possessed enhanced cell penetration ability. We postulated that TAT could be guided to an intercellular level, at which point we would expect that it acts as a possible inhibitor of Plk1 PBD. The ability of TAT-PLHSpT to inhibit proliferation in a variety of cancer cells grown in culture was evaluated. Anticancer effects of TAT conjugation in the cancer cells were enhanced in a dose-dependent manner compared to controls of only TAT and PLHSpT.

To evaluate the detailed mechanism of TAT-PLHSpT in cancer cells, the assays were developed to measure the Plk1 kinase activity in the cell in response to the peptide treatment. Our guess was that the uptake of PLHSpT into the nucleus may induce the inhibition of Plk1 kinase activity, playing an important role in mitosis. Thus, we tested the Plk1 kinase activity in cancer cell lines HeLa, HT-29, and KB after stimulation with TAT, PLHSpT, and TAT-PLHSpT. The PLK-1 kinase activity was statistically reduced after stimulation for 24 and 48 h with TAT-PLHSpT, when compared to TAT- and PLHSpT-treated groups (Fig. 5). In HeLa and HT-29 cells, the inhibition rate of Plk1 kinase activity was reduced to a greater extent in samples stimulated for 48 h than 24 h. The rate of Plk1 kinase activity after stimulation with TAT-PLHSpT showed that HeLa showed about 60 % at 24 h and 41.5 % at 48 h; HT-29, 55.3 % at 24 h and 40.2 % at 48 h; and KB, 44.5 % at 24 h and 50.5 % at 48 h. TAT-PLHSpT treatment group was only shown to inhibit the Plk1 kinase activity in cancer cells, which means that TAT-PLHSpT exhibits specific and selective interaction with Plk1.

We also performed immunofluorescence staining to demonstrate that PLHSpT induced the inhibition of cell differentiation. The images collected showed that the cell division step was delayed by the activation of TAT-PLHSpT such that the nuclei of tumor cells displayed multipolar spindles and misaligned chromosomes (Fig. 6). On the other hand, the inhibition effect of cell mitosis by PLHSpT was not induced in tumor cells, just as the inhibition effect was not clear in the control sample.

For further explanation of the reasons behind the induction of cancer cell death with TAT-PLHSpT treatment, we performed cell cycle analysis and measured apoptosis by immunofluorescence, since Plk1 expression and activity are highly linked to mitosis. We confirmed the apoptotic effect of TAT-PLHSpT on HT-29 and KB tumor cells by AO/EtBr staining (Fig. 7), and on HeLa cells with a western blot assay using PARP and caspase-3 (Fig. 8). AO/EtBr staining revealed that TAT-PLHSpT induced cell apoptosis in HT-29 and KB cells compared to TAT- and PLHSpT-treated groups, where the induction of cancer cell apoptosis was not clear (Fig. 7). Furthermore, the expression of apoptosis-related factors (caspase-3 and PARP cleaved form) was significantly increased in the TAT-PLHSpT treated group. The expression of caspase-3 and PARP cleaved form was the highest in the control incubated with TAT-PLHSpT for 48 h (Fig. 8). In the cell cycle study, TAT-PLHSpT was observed to cause mitotic arrest and chromosome congression defects. The post-TAT-PLHSpT treatment morphological images also showed significant changes (data not shown here). Moreover, the peptide induced cell death by apoptosis in both HT-29 and KB cells.

Conclusions

We synthesized a new specific targeting peptide of Plk-1 PBD, namely, TAT-PLHSpT, which can easily penetrate into the cell using cell-permeable peptide conjugation. The peptide not only inhibited cancer cell proliferation by blocking mitosis, but also induced cancer cell death. This study demonstrated that TAT-PLHSpT is a potential anticancer agent. Further studies are warranted to investigate the antitumor effect of the TAT-PLHSpT in vivo using tumor-bearing animal models.

Acknowledgments This research was supported in part by the Grant from Korea Basic Science Institute (T33418) and by a National Research Foundation of Korea (NRF) Grant funded by the Korean government (MEST) (2012-0006388).

Conflict of interest The authors declare that they have no competing financial interests.

References

Barr FA, Sillje HH, Nigg EA (2004) Polo-like kinases and the orchestration of cell division. Nat Rev Mol Cell Biol 5(6):429–440. doi:10.1038/nrm1401

Cheng KY, Lowe ED, Sinclair J, Nigg EA, Johnson LN (2003) The crystal structure of the human polo-like kinase-1 polo box domain and its phospho-peptide complex. EMBO J 22(21):5757–5768. doi:10.1093/emboj/cdg558

Dai W (2005) Polo-like kinases, an introduction. Oncogene 24(2):214–216. doi:10.1038/sj.onc.1208270

de la Fuente JM, Berry CC (2005) Tat peptide as an efficient molecule to translocate gold nanoparticles into the cell nucleus. Bioconjugate chem 16(5):1176–1180. doi:10.1021/bc050033+

Elia AE, Cantley LC, Yaffe MB (2003a) Proteomic screen finds pSer/pThr-binding domain localizing Plk1 to mitotic substrates. Science 299(5610):1228–1231. doi:10.1126/science.1079079

Elia AE, Rellos P, Haire LF, Chao JW, Ivins FJ, Hoepker K, Mohammad D, Cantley LC, Smerdon SJ, Yaffe MB (2003b) The molecular basis for phosphodependent substrate targeting and regulation of Plks by the Polo-box domain. Cell 115(1):83–95

Fawell S, Seery J, Daikh Y, Moore C, Chen LL, Pepinsky B, Barsoum J (1994) Tat-mediated delivery of heterologous proteins into cells. Proc Natl Acad Sci USA 91(2):664–668

Ferrari A, Pellegrini V, Arcangeli C, Fittipaldi A, Giacca M, Beltram F (2003) Caveolae-mediated internalization of extracellular HIV-1 tat fusion proteins visualized in real time. Mol Ther 8(2):284–294. pii:S1525001603001229

Gumireddy K, Reddy MV, Cosenza SC, Boominathan R, Baker SJ, Papathi N, Jiang J, Holland J, Reddy EP (2005) ON01910, a non-ATP-competitive small molecule inhibitor of Plk1, is a potent anticancer agent. Cancer Cell 7(3):275–286. doi:10.1016/j.ccr.2005.02.009

Gump JM, Dowdy SF (2007) TAT transduction: the molecular mechanism and therapeutic prospects. Trends Mol Med 13(10):443–448. pii:S1471-4914(07)00164-5/j.molmed.2007.08.002

Josephson L, Tung CH, Moore A, Weissleder R (1999) High-efficiency intracellular magnetic labeling with novel superparamagnetic-Tat peptide conjugates. Bioconjugate chem 10(2):186–191. doi:10.1021/bc980125h

Kim SM, Yoon S, Choi N, Hong KS, Murugan RN, Cho G, Ryu EK (2012) In vivo tumor imaging using polo-box domain of polo-like kinase 1 targeted peptide. Biomaterials 33(29):6915–6925. doi:10.1016/j.biomaterials.2012.06.046

Lansing TJ, McConnell RT, Duckett DR, Spehar GM, Knick VB, Hassler DF, Noro N, Furuta M, Emmitte KA, Gilmer TM, Mook RA Jr, Cheung M (2007) In vitro biological activity of a novel small-molecule inhibitor of polo-like kinase 1. Mol Cancer Ther 6(2):450–459. doi:10.1158/1535-7163.MCT-06-0543

Lenart P, Petronczki M, Steegmaier M, Di Fiore B, Lipp JJ, Hoffmann M, Rettig WJ, Kraut N, Peters JM (2007) The small-molecule inhibitor BI 2536 reveals novel insights into mitotic roles of polo-like kinase 1. Curr bio 17(4):304–315. doi:10.1016/j.cub.2006.12.046

Lewin M, Carlesso N, Tung CH, Tang XW, Cory D, Scadden DT, Weissleder R (2000) Tat peptide-derivatized magnetic nanoparticles allow in vivo tracking and recovery of progenitor cells. Nat Biotechnol 18(4):410–414. doi:10.1038/74464

Li L, Wang X, Chen J, Ding H, Zhang Y, Hu TC, Hu LH, Jiang HL, Shen X (2009) The natural product Aristolactam AIIIa as a new ligand targeting the polo-box domain of polo-like kinase 1 potently inhibits cancer cell proliferation. Acta Pharmacol Sin 30(10):1443–1453. doi:10.1038/aps.2009.141

Liu F, Park JE, Qian WJ, Lim D, Graber M, Berg T, Yaffe MB, Lee KS, Burke TR Jr (2011) Serendipitous alkylation of a Plk1 ligand uncovers a new binding channel. Nat Chem Biol 7(9):595–601. doi:10.1038/nchembio.614

Nagahara H, Vocero-Akbani AM, Snyder EL, Ho A, Latham DG, Lissy NA, Becker-Hapak M, Ezhevsky SA, Dowdy SF (1998) Transduction of full-length TAT fusion proteins into mammalian cells: TAT-p27Kip1 induces cell migration. Nat Med 4(12):1449–1452. doi:10.1038/4042

Pooga M, Soomets U, Hallbrink M, Valkna A, Saar K, Rezaei K, Kahl U, Hao JX, Xu XJ, Wiesenfeld-Hallin Z, Hokfelt T, Bartfai T, Langel U (1998) Cell penetrating PNA constructs regulate galanin receptor levels and modify pain transmission in vivo. Nat Biotechnol 16(9):857–861. doi:10.1038/nbt0998-857

Reindl W, Yuan J, Kramer A, Strebhardt K, Berg T (2008) Inhibition of polo-like kinase 1 by blocking polo-box domain-dependent protein–protein interactions. Chem Biol 15(5):459–466. doi:10.1016/j.chembiol.2008.03.013

Richard JP, Melikov K, Vives E, Ramos C, Verbeure B, Gait MJ, Chernomordik LV, Lebleu B (2003) Cell-penetrating peptides. A reevaluation of the mechanism of cellular uptake. J Biol Chem 278(1):585–590. doi:10.1074/jbc.M209548200

Schwarze SR, Hruska KA, Dowdy SF (2000) Protein transduction: unrestricted delivery into all cells? Trends Cell Biol 10(7):290–295

Shibagaki N, Udey MC (2002) Dendritic cells transduced with protein antigens induce cytotoxic lymphocytes and elicit antitumor immunity. J Immunol 168(5):2393–2401

Strebhardt K (2010) Multifaceted polo-like kinases: drug targets and antitargets for cancer therapy. Nat Rev Drug Discovery 9(8):643–660. doi:10.1038/nrd3184

Strebhardt K, Ullrich A (2006) Targeting polo-like kinase 1 for cancer therapy. Nat Rev Cancer 6(4):321–330. doi:10.1038/nrc1841

Torchilin VP, Rammohan R, Weissig V, Levchenko TS (2001) TAT peptide on the surface of liposomes affords their efficient intracellular delivery even at low temperature and in the presence of metabolic inhibitors. Proc Natl Acad Sci USA 98(15):8786–8791. doi:10.1073/pnas.151247498

Vives E (2003) Cellular uptake [correction of utake] of the Tat peptide: an endocytosis mechanism following ionic interactions. J Mol Recogn 16(5):265–271. doi:10.1002/jmr.636

Vives E, Brodin P, Lebleu B (1997) A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. J Biol Chem 272(25):16010–16017

Watanabe N, Sekine T, Takagi M, Iwasaki J, Imamoto N, Kawasaki H, Osada H (2009) Deficiency in chromosome congression by the inhibition of Plk1 polo box domain-dependent recognition. J Biol Chem 284(4):2344–2353. doi:10.1074/jbc.M805308200

Wender PA, Mitchell DJ, Pattabiraman K, Pelkey ET, Steinman L, Rothbard JB (2000) The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: peptoid molecular transporters. Proc Natl Acad Sci U S A 97(24):13003–13008. doi:10.1073/pnas.97.24.13003/24/13003

Yun SM, Moulaei T, Lim D, Bang JK, Park JE, Shenoy SR, Liu F, Kang YH, Liao C, Soung NK, Lee S, Yoon DY, Lim Y, Lee DH, Otaka A, Appella E, McMahon JB, Nicklaus MC, Burke TR Jr, Yaffe MB, Wlodawer A, Lee KS (2009) Structural and functional analyses of minimal phosphopeptides targeting the polo-box domain of polo-like kinase 1. Nat Struct Mol Biol 16(8):876–882. doi:10.1038/nsmb.1628