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The PEP-1 Recombination Protein Provide a Promising Strategy for the Biomacromolecules Delivery in Thp-1 and LSK Cells

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Abstract

CPPs as known as cell-penetrating peptides, can transport the biomacromolecules by non-covalent package or convalent binds. With high efficiency and low toxicity, the CPPs were widely investigated in research and clinical trials and regarded as a potential strategies for disease therapy. The LSKs and Thp-1 cells are classical research models. However, the traditional biomacromolecules expression or delivery was performed by virus infection or materials packge, which may limited by molecule size, cell stress response or the cost. In our study, we purified a CPP recombination protein PEP-1-GFP and test the efficiency of penetrating into the LSK and Thp-1 cells. The PEP-1-GFP could permeated in Thp-1 cells in short time at a safety concentration, but showed a compromising efficiency in LSK cells in spite of the efficiency increasing as the condition improvement. These results suggested that the CPP PEP-1 would act as a promising transporter for the biomacromolecules delivery in these cells.

Keywords

PEP-1; CPP; Thp-1; LSK.

1. Introduction

Biomacromolecule delivery has been greatly investigate in research and clinical since recombinant human insulin firstly approved by the FDA for the treatment of diabetes in 1980s [1]. As most of the water-soluble macromolecules can't pass the cell membrane freely, several strategies include virus infection, material packaging and cell-penetrating peptides (CPPs) present promising prospect to improve the delivery efficiency of these molecules [2-5]. The use of these strategies are often influenced by immunogenicity, cytotoxicity and cost. Hematopoietic stem/progenitor cells (HSC/HPCs) and Thp-1 cell are important cell modes in research of blood system [6,7]. However, the efficiency of biomolecules expression by virus infection are seriously affected by molecular size. And the virus infection often induced immunological stress which may impact the function of HSCs and the polar of Thp1 cells. Moreover, the screen of positive cells after virus infection needs more than 24h, which may lead to the differential of HSCs and decline of stemness and function if culture in vitro for that long time [8]. The packaging materials with low toxicity and high delivery efficiency are often limited in cost, especially at experiment in vivo. Thus, the scheme for the biomacromolecule delivery in these cells needs further improved.

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CPPs are a kind of short oligopeptides with positive charge, consisting of 5 to 30 amino acids, which can loading various target molecules by covalent/non-covalent binding, and crossing biofilms [9]. Known for the high transduction efficiency and low cytotoxicity, CCPs have received extensive attention in research and clinical trials [2,10]. PEP-1 is one of the CPPs, containing a N-terminal hydrophobic motif, a C-terminal hydrophilic motif (NLS) and a linker which improves the flexibility and the integrity of N- and C-terminal domains [11]. Pep-1 has been used effectively in a variety of cell lines. In this study, we use the PEP-1-GFP recombination protein to incubate with LSKs (Lin-Scal1+C-kit+, the main populations of hematopoietic stem/progenitor cells) and Thp-1 cells to test the efficiency of delivery, providing a promising strategies for the non-engineered biomacromolecule delivery strategies for these cells.

2. Materials and Method

2.1 Plasmid Construction and Protein Purification

The PEP1 sequence as 5'- ATGAAAGAAACCTGGTGGGAAACCTGGTGGAC CGAATGGTCTCAGCCGAAGAAGAAACGTAAAGTG-3', which peptide as MKETWWETWWTEWSQPKKKRKV, were cloned with GFP-5xHis into PET28b (Addgene). The PET28b-PEP-1-GFP was transformed in to E.coli strain BL21(DE3) (TransGen Biotech). The positive clones were culture overnight and induced protein expression with 800 μM IPTG (SANGO) in 37 ℃ for 4h. Pelleted the bacteria by centrifugation, digested by lysozyme (1 mg/ml) with PMSF in lysis buffer (20 mM PBS with 0.5 M NaCl). Sonicated the suspension, filtrated by 0.45 μM filter and purified with nickel column (SANGO). The washing solution contained 20 mM PBS, 0.5M NaCl with 20mM imidazole. The final elution, containing 500 mM imidazole (Sigma) in lysis buffer, was exchanged buffer to PBS by ultrafiltration tube (Millipore).

2.2 Cell Line Culture and Primary Cell Isolation

The Thp-1 cells (ATCC) were cultured in RPMI-1640 (Gibco) with 10% FBS (Gibco) and 1% P/S (Gibco). The LSK (Lin-Scal1+C-kit+, main population of hematopoietic stem/progenitor cells) were isolated from bone marrow cells of C57BL6J mice by Aria3 (BD). The cells were staining and gating as Lin-Scal1+C-kit+ cells from mice bone marrow.

2.3 Protein Quantification, Cell Incubation and Microexamination

The purified protein was quantified by page electrophoresis combined coomassie brilliant blue (Beyotime) staining, and BCA quantification kit (Thermo Fisher). The protein was incubation with cells at specified temperature with time. Then observed and acquired by fluorescence microscope (Carl Zeiss).

2.4 Statistical Analysis

Two-tailed unpaired student's t-test was used for statistical analysis by Graphpad Prism 8. The error bars represent the mean ± SEM of three more independent repeats (**** $P<0.0001$).

3. Result

3.1 Expression and Purification of PEP-1-GFP

The PEP-1 contained a N-terminal tryptophan-rich domain, a C-terminal NLS domain and a linker domain. We clone the PEP-1 coupled with GFP-5xHis in PET28b and transformed in BL21 (Figure 1A). Positive clones were screened and 8 clones were induced protein expression (Figure 1B). The Clone 2 showed the highest expression efficiency and this clone was used for the further purification. The lysis solution of clone 2 were loading to the nickel column and the impurity proteins were washed out by lysis buffer and washing solution. The final elution sample were concentrated and exchanges buffer to PBS by ultrafiltration tube. The abundance of the PEP-1-GFP in the enriched sample was about 80% and the final concentration of PEP-1-GFP was about 30μM.

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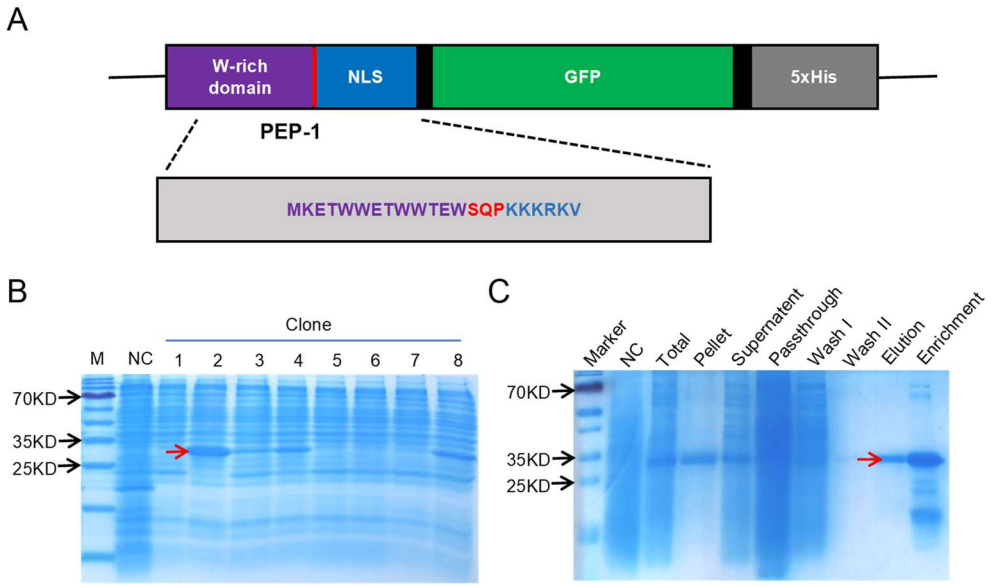

Figure 1. PEP-1-GFP construction and protein purification

A. The schematic figure of plasmid construction. B. The 8 clones carried PET-28b-PEP-1-GFP were selected, cultured and induced protein expression. Ran the PAGE electrophoresis and staining by coomassie brilliant blue. The uninduced clone were used as negative control (NC). C. The Clone 2 was used for protein purification. The suspension (total) , the supernatant and pellet after centrifugation were used as purification control. The Wash I was using lysis buffer, Wash II was using lysis buffer with 20 mM imidazole. The enrichment samples was quantified by BCA protein quantification kit, the concentration was about 30 μM (this sample was diluted as 1:20 for this PAGE analysis).

3.2 PEP-1-GFP Permeated in Thp-1 Cells with Concentration Dependence in Efficiency

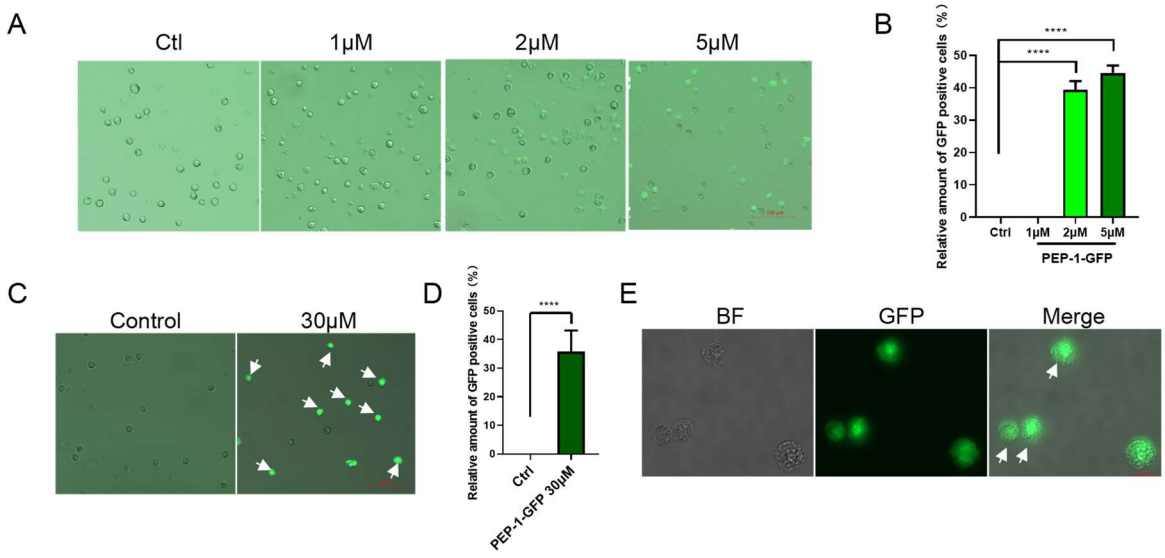

Figure 2. PEP-1-GFP penetrated in Thp-1 cells after short time incubation.

A. The Thp-1 cells incubated with PEP-1-GFP at room temperature for 2 h. The GFP positive cells were indicated by white arrows. Scale bars: 100 μm. B. The quantification of A. C. The Thp-1 cells incubated with PEP-1-GFP (30 μM) at room temperature for 30min. The GFP positive cells were indicated by white arrows. Scale bars: 100 μm. D. The quantification of C. E. The PEP-1-GFP accumulated in nucleus. BF: bright field. Scale bars: 20 μm.

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The Thp-1 cells were harvest and washed by PBS for twice. The PEP-1-GFP was diluted and incubated with Thp-1 2h at room temperature (Figure 2A and B). As the concentration higher, the ratio of the GFP positive increased significantly, suggesting that we could reach a high efficiency (above 40%) by using a higher concentration of PEP-1-GFP and shorter time which may reduce the stress of cells during incubation in vitro. Thus, we incubated Thp-1 cells with 30 μM PEP-1-GFP for 30min at room temperature. The GFP positive cells (about 35%) was comparable with the 2h incubation with 5 μM, and the fluorescence intensity of GFP was much stronger, consistent with our assumption. In addition, PEP-1-GFP accumulated more in the nuclear in some of the cells, making the possibility of specific regulation nucleus by CPPs transportation.

3.3 PEP-1-GFP Could Pass in LSK

The bone marrow cells were obtained from the legs of sacrificed mice. The gating strategy was performed as in Figure 3A, and only the Lin-Scal1+C-kit+ cells were sorted, as LSK cells. The LSK cells were washed by PBS with 2% FBS, and incubated with PEP-1-GFP as the condition showing in Figure 3B. The GFP positive LSK cells showed significantly increase in the group with 30 μM or 37 ℃ or 1 h incubation. These suggest that the higher concentration and temperature with longer incubation would be helpful for the PEP-1-GFP passed in LSK cells. However, the efficiency is not as good as in Thp-1 cells (less than 20%). In addition, the LSK cells isolated from the bone marrow must be treated carefully in vitro. As longer time out of the niche or higher incubation temperature may reduce the viability and function of LSK cells, incubating LSK cells with 30μM PEP-1-GFP at 37 ℃ for 1 h (the highest efficiency group) not only showed a compromised efficiency compared with Thp-1, but also increase the risk for decrease the functions of LSK cells.

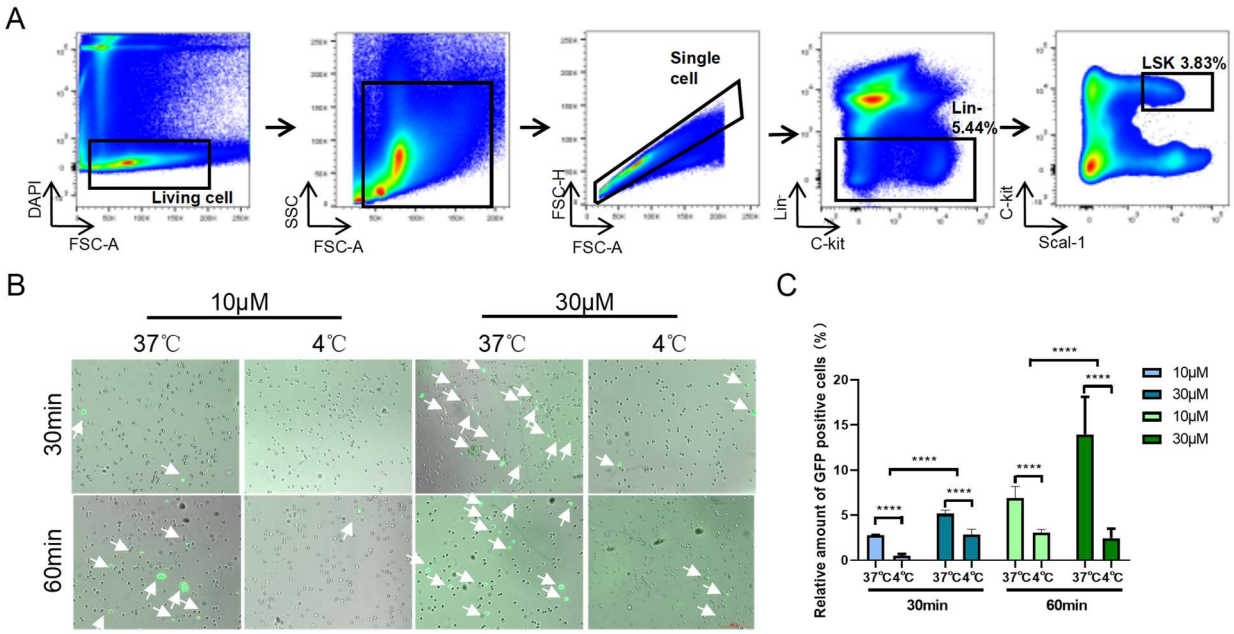

Figure 3. PEP-1-GFP could penetrate in LSK cells.

A. The schematic figures for sorting LSK cells from bone marrow cells by Aria3. B. The LSK cells incubated with PEP-1-GFP at indicated conditions. The GFP positive cells were indicated by white arrows. Scale bars: 100 μm. C. The quantification of B.

4. Conclusion

The biomacromolecular therapeutics show promising in different diseases. Recombinant protein with CPP tag could successfully deliver the in cells, animal models and human trials. The biophysical characteristics as charge, shape, amphipathicity, structures of biomacromolecule influence the entry of CPP-protein into the cells. As a well investigated CPP, the PEP-1 has been proved to successfully

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transport proteins in different cells without toxicity and transport proteins to nucleus by the non-covalent package. It worth nothing to say, almost no toxicity was detected when the Pep-1 reach to 100 μM, and only 10% cell viability decreased when cells exposed to 1 mM PEP-1. Thus, the concentration we used supposed to be no harmful for the cells.

In our study, we test the penetration efficiency PEP-1-GFP recombination protein at 10 μM and 30 μM in Thp-1 and LSK cells. The PEP-1-GFP could pass into Thp-1 efficiently at room temperature for 30min incubation and accumulated in the nucleus, which suggest a high potency for the biomacromolecule delivery in this cell. And the PEP-1-GFP performed a lower efficiency even at 30μM incubation for 1h at 37℃ in LSK cells. Thus, further improvement for the delivery should be investigate. In spite of the PEP-1 shows low toxicity, the PEP-1 recombination protein may induce immune response in different condition depends on the protein and cells type. As the PEP-1 could be used in protein vaccination , the cell response of Thp-1 and LSK remains further investigation.

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