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A protein delivery system using 30Kc19 cell-penetrating protein originating from silkworm

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ABSTRACT

Cell-penetrating protein and its protein transduction domain have been used to deliver drugs and proteins into the cells via receptor-independent endocytosis. A number of cell-penetrating proteins including TAT derived from HIV-1 virus, VP22 from herpes simplex virus and Antennapedia from *drosophila* have been discovered. Here, we report a cell-penetrating protein, 30Kc19, originating from the hemolymph of silkworm, *Bombyx mori*. The 30Kc19 is the first cell-penetrating protein that has been found in insect hemolymph. When the 30Kc19 protein produced from recombinant *Escherichia coli* was supplemented into the medium for mammalian cell culture, 30Kc19 efficiently penetrated into various types of cells and localized at subcellular compartments including mitochondria and cytoplasm. 30Kc19 also delivered cargo proteins such as green fluorescence protein into the cells even though cargo proteins are not able to penetrate into cells by themselves. In addition to the *in vitro* system, 30Kc19 exhibited the protein transduction property *in vivo*. When 30Kc19 was intraperitoneally injected into mice, 30Kc19 delivered cargo proteins into various organ tissues of model animals without producing toxicity. Therefore, 30Kc19 has a great potential as a cell-penetrating protein that can be used as a medicinal tool to deliver cargo molecules including proteins into the target organ tissues in the body.

1. Introduction

Cell-penetrating proteins are a group of proteins that penetrate living cells via a receptor-independent pathway. Cell-penetrating proteins and their protein transduction domain (PTD) have been used to deliver functional macromolecules, such as proteins [1,2], nucleic acids [3,4] and nanoparticles [5–7], into cells [8,9]. In 1988, Green and Frankel independently demonstrated the ability of the HIV-1 (human immunodeficiency virus-1) derived-TAT protein to penetrate into the cells in a receptor-independent, concentration-dependent manner [10,11]. Since then, several proteins and peptides have been found to move across the cell membrane through a similar mechanism. The *Drosophila* homeoprotein Antennapedia (Antp), a herpes simplex virus structural protein VP22 (VP22), and the HIV-1 transcriptional activator TAT protein (TAT) are the best representatives of this class of proteins [12–18].

Here, we report a cell-penetrating protein, 30Kc19, originating from the hemolymph of silkworm, *Bombyx mori*. 30Kc19 is a member of the 30K protein family (30Kc6, 30Kc12, 30Kc19, 30Kc21 and 30Kc23), which is a family of similar structured proteins found in silkworm hemolymph that have molecular weights around 30 kDa [19,20]. These proteins are synthesized in fat body cells and accumulate in the hemolymph during the final instar (5th instar) larvae and the early pupal stage [21,22]. During metamorphosis from larvae to pupae, the 30K proteins are transferred from the hemolymph to fat body cells where they are deposited [23,24]. The biological functions of the 30K proteins in silkworms have not been fully determined, although several studies have recently examined their functional properties [24,25].

In our previous studies, we demonstrated that 30K proteins inhibited apoptosis in various animal cells [26–31]. Cellular apoptosis was inhibited not only by *30K gene* expression but also by the addition of recombinant 30K protein produced from *Escherichia coli* into the culture medium. 30Kc19 is a major protein among the 30K proteins. In this work, we investigated the cell-penetrating properties of 30Kc19 protein.

2. Materials and methods

2.1. Plasmid construction and production of 30Kc19 and GFP-30Kc19

Total RNA was isolated from the silkworms, fifth-instar larvae using RNeasy (Qiagen, Valencia, CA, USA) and the 30Kc19 cDNA was obtained from total RNA by

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