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Review

Baculovirus for gene delivery to mammalian cells: Past, present and future

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ABSTRACT

Baculovirus is an insect virus which has been used for more than thirty years for production of recombinant proteins in insect cells. However, baculovirus can also be harnessed for efficient gene delivery to mammalian cells if it is equipped with mammalian promoters. This technology is known as BacMam and has been used for gene delivery to immortalized cell lines, stem cells, and primary cells, as well as for gene delivery in animals. Baculovirus has unique features when compared to mammalian viruses. Besides the fact that it is replication-incompetent and does not integrate into the host genome, it has large capacity for foreign DNA. This capacity can for example be used to deliver multiple genes for reprogramming of stem cells, or for delivery of large homology constructs for genome editing. In this review, we provide a brief overview of baculovirus-based gene delivery and its recent applications in therapy and basic research. We also describe how baculovirus is manipulated for efficient transduction in mammalian cells and we highlight possible future improvements.

1. Introduction

The baculovirus/insect cell system has been widely used in many academic and industrial laboratories for production of functional recombinant proteins (Kost et al., 2000; O'Reilly et al., 1994; Smith et al., 1983). Indeed, this system has some features that makes it unique as a protein expression system. Compared to bacteria it offers better folding of mammalian proteins (especially extracellular proteins), accurate posttranslational modifications, e.g., glycosylation, and the ability to produce protein complexes. Also, in comparison to mammalian expression systems it offers higher expression levels (Assenberg et al., 2013; Fernandes et al., 2013; van Oers et al., 2015). These high expression levels are based on the ability of the virus to take over infected cells in the late phase. In this phase, nearly all endogenous promoters are shut down and transcription mainly occurs from the viral polyhedrin and p10 promoters (Weyer and Possee, 1991). These two promoters are nowadays mainly used in commercial expression systems. So far, a tremendous number of properly folded proteins or protein complexes (e.g., antibodies (Guttieri and Liang, 2004), enzymes (Hodoniczky et al., 2005), hormones (Kim et al., 2005), kinases (Gao et al., 2005), membrane proteins (Trometer and Falson, 2010), multiprotein complexes (Berger et al., 2004), and virus like particles (Fernandes et al., 2013)) from different organisms have been produced using baculovirus/insect cell system. For example, Cervarix, a virus-like particle vaccine against human papillomavirus types 16 and 18 (from GSK), was produced using baculovirus/insect cells system. This product is approved by European Medicine Agency (EMEA) and FDA (Harper et al., 2004). However, in the mid-1990s, new possibilities for the baculovirus system were identified. Two groups equipped baculovirus with mammalian promoters and efficiently transduced vertebrate cells (Boyce and Bucher, 1996; Hofmann et al., 1995). This system was called “BacMam” and has been used for many in vitro to in vivo applications. BacMam has interesting features for gene delivery that differentiate it from other viral and non-viral gene delivery approaches (Airenne et al., 2013; Kost et al., 2005; Uef, 2011; van Oers et al., 2015). Baculovirus is a non-integrating virus, with a large transgene capacity (> 100kbp), and does not replicate in mammalian cells (Mudgal et al., 2013). Also, baculovirus transduces a variety of cell types including both dividing and non-dividing cells, and shows a low cytotoxicity in vertebrate cells (Airenne et al., 2013). Emerging new applications in cell biology and drug development require gene delivery systems that have a high level of safety in delivery, flexibility in size of cargo, and simplicity in production. For example, gene therapy approaches in humans, of which there have been an increasing number in recent years, might be used successfully to treat cancer. For example, Kymriah™, a commercial preparation of T cells expressing a chimeric antigen receptor, calls for vehicles with a high level of safety and efficacy in gene delivery (Dunbar et al., 2018). Kymriah is based on a retrovirus, but an engineered baculovirus with improved features may fulfil these demands in future.

In this review, we first briefly describe the history of baculovirus biology. Then, important papers which used the BacMam system for

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delivery of either single or multiple gene to mammalian cells are highlighted. Next, we focus on recent in vitro, ex vivo and in vivo applications of the BacMam system in therapy and basic research (see (Mansouri and Berger, 2018) for a more comprehensive list of applications). Then, approaches for improvement of the virus are discussed. Finally, we highlight future directions toward an optimized baculovirus.

2. Baculovirus biology

Baculoviruses are a diverse group of DNA viruses capable of infecting hundreds of insect species (Chen et al., 2011). Baculovirus normally infects and ultimately kills insect cells (van Oers et al., 2015). Therefore, these viruses are also used as biological pesticides to protect crops from insects (Summers, 2006). Their genomes are circular double-stranded DNA, with a size of 80 to 180 kbp, and are packed in a rod-shaped nucleocapsid surrounded by a lipid envelop. Typically baculoviruses are 40–50 nm in diameter and 200–400 nm in length (Ihalainen et al., 2010). *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is the best studied and most extensively used baculovirus (van Oers, 2011). It was initially identified in 1971, and the consensus sequence of the AcMNPV genome was published in 1982 (Vail et al., 1971; Vlak and Smith, 1982). These pioneering studies were the prerequisite for the first AcMNPV-based baculovirus expression system (Smith et al., 1983). Thus, baculovirus discussed here refers to AcMNPV unless otherwise mentioned. AcMNPV is characterized by having two different virion cycle types, budded virions (BVs) and occlusion derived virus (ODV). BV bud from the cell membrane and spread infection within the host, whereas ODVs gain their envelope from the nuclear membrane and spread infection between hosts (Federici, 1997; Funk et al., 1997). In vitro expression systems work with the BV type. Exact mechanism and the receptor(s) on the host cell surface which mediate AcMNPV cell entry are not completely understood (Airenne et al., 2011). However, mammalian cell surface components like heparin sulfate, proteoglycans, phospholipids, and integrins are receptor candidates for AcMNPV (Airenne et al., 2013). Interaction between cell surface receptors and AcMNPV major envelope proteins, e.g., gp64, triggers attachment of the virus to the cell. After that, viruses are endocytosed and the associated change of pH inside early endosomes stimulates conformational change of gp64 which result in fusion of the viral envelope with the membrane of early endosomes. This fusion releases the nucleocapsid to the cytoplasm of host cells. There the nucleocapsid translocates to the nucleus through the nuclear pore complex and releases its genome within the nucleus (Airenne et al., 2011; Kataoka et al., 2012; Long et al., 2006).

3. Baculovirus for gene delivery to mammalian cells

After successful baculovirus-based protein production in insect cells, the question was raised whether baculovirus can infect and subsequently deliver genes to mammalian cells. Earlier studies showed that baculovirus is able to enter non-target vertebrates (Gröner et al., 1984; Volkman and Goldsmith, 1983) and also some uncertain observations suggested baculovirus driven gene expression in mammalian cells (Carbonell et al., 1985; Carbonell and Miller, 1987). In 1995, Hofmann and colleagues used a baculovirus harboring a CMV-driven *LacZ* gene to express ß-galactosidase in human and rabbit hepatocytes (Hofmann et al., 1995). In 1996, Boyce and colleagues also confirmed efficient baculovirus delivery and expression of ß-galactosidase under control of a RSV promoter in different mammalian cell types such as HepG2 and primary hepatocytes (Boyce and Bucher, 1996). These two pioneering studies attracted attention to baculovirus as a potential vector for gene delivery. Since then, many groups in academia and the pharmaceutical industry have used baculovirus as a robust gene delivery system for a wide variety of cells including cell lines, e.g., HEK293, COS7, CHO, and BHK, stem cells e.g., ESCs and iPSCs, and primary cells, e.g., HUVEC, rat embryo fibroblasts (REF), and neurons, (Condreay et al., 1999; Kost and Condreay, 2002; Mansouri et al., 2016). Here, we revisit some important points that should be considered when BacMam is chosen as gene delivery system. First, BacMam technology requires a mammalian promoter. A variety of promoters with different stringency and efficiency are available for this purpose (Qin et al., 2010). Tissue-specific promoters allow expression of the transgene only in desired cells within a mixture of cell populations or tissues (Zheng and Baum, 2008). Also, some common promoters (e.g., CMV) are either inactive or have minimal activity in some cell types, e.g., ESCs, (Sinn et al., 2017). Therefore, the mammalian promoter should be carefully chosen based on the target cell type. Second, it has been shown that baculovirus is able to transduce different mammalian cell types. However, baculovirus doesn't transduce all mammalian cells with the same efficiency. For example, a low level of transduction was reported in cells of hematopoietic origin (Schauber et al., 2004). However, there are strategies to improve tropism of the virus and to increase transduction efficiency (see the next part). Third, BacMam is considered to be a transient expression system. Nevertheless, addition of some elements, e.g., antibiotic resistance gene, transposon sequences, permits generation of stable cell lines from transduced cells (Condreay et al., 1999; Luo et al., 2012; Turunen et al., 2014). Fourth, it has been shown that addition of histone deacetylase inhibitors, e.g. sodium butyrate, can increase levels of gene expression in transduced mammalian cells (Sarkis et al., 2000). Although, this method is helpful for protein production in cells, it should be considered that these inhibitors usually have toxic effects on mammalian cells and can change the cellular behavior. Fifth, generation of baculovirus takes place in insect cells at 27 °C (Sung et al., 2014). Therefore, access to special cell culture equipment is required for baculovirus production.

Nowadays, production of baculovirus for BacMam can be done by introducing mammalian expression cassettes in commercially available vectors for insect cells. For example, pFastBac (Invitrogen) is one of these vectors. This vector also contains Tn7L/Tn7R sequences which are essential for transposition of the recombinant plasmid (carrying expression cassette) into the baculovirus genome (Bac-to-Bac, Invitrogen). Nevertheless, other published baculovirus systems are also suitable for this purpose. See (Lin et al., 2011) for an overview of available systems.

One of the most interesting features of baculovirus which gives it an

Table 1
Comparison between baculovirus and the other viruses for gene delivery to mammalian cells.

	Baculovirus		Other viruses (e.g., retro-, lenti-, adeno-associated-, adenoviruses)
Pros	- Large cargo capacity - High transduction efficiency in both dividing and non-dividing - Non-integrating virus - BSL1 cell culture facility for virus handling	Cons	- Small cargo capacity (usually below 10 kb) - Inability to transduce non-dividing cells (except retroviruses) - Integrating viruses (retro- and lentiviruses) - BSL2 cell culture facility for virus handling
Cons	- Long and time-consuming process for virus production - Transient gene expression - Need for extra equipment for insect cell cultures	Pros	- Fast and simple process for virus production - Stable gene expression (except AAV and adenoviruses) - No need for extra equipment - FDA approved for gene delivery to human