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Baculovirus as a gene delivery vector: Recent understandings of molecular alterations in transduced cells and latest applications

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ABSTRACT

Baculovirus infects insects in nature and is non-pathogenic to humans, but can transduce a broad range of mammalian and avian cells. Thanks to the biosafety, large cloning capacity, low cytotoxicity and non-replication nature in the transduced cells as well as the ease of manipulation and production, baculovirus has gained explosive popularity as a gene delivery vector for a wide variety of applications. This article extensively reviews the recent understandings of the molecular mechanisms pertinent to baculovirus entry and cellular responses, and covers the latest advances in the vector improvements and applications, with special emphasis on antiviral therapy, cancer therapy, regenerative medicine and vaccine.

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1. Introduction

Baculoviruses are a diverse group of DNA viruses capable of infecting more than 600 insects, among which Autographa californica multiple nucleopolyhedrovirus (AcMNPV) is the best characterized and most extensively employed, thus baculovirus discussed in this article refers to AcMNPV unless otherwise noted. AcMNPV contains a circular dsDNA genome of ≈134 kb and replicates in a bi-phasic fashion. The viral proteins polyhedrin and p10 are expressed abundantly in infected cells and are dispensable for virus replication, thus recombinant baculovirus can be constructed by placing the foreign gene under the control of polyhedrin or p10 promoter, and used to infect insect cells for foreign gene expression. Such baculovirus–insect cell expression system has been exhaustively utilized for the production of numerous recombinant proteins (Kost and Con dreay, 1999) and commercial vaccine products such as Cervarix® and Provenge® (Hitchman et al., 2009; Lin et al., 2011).

In addition to insect cells, baculovirus is able to transduce animal cells of human, rodent, rabbit, porcine, bovine, fish and avian origins (Hu, 2005, 2006) as well as relatively primitive cells including embryonic stem cells, adult stem cells (Table 1) and induced pluripotent stem cells (Fig. 1). Within the baculovirus–transduced cells, the transgene can be expressed as long as it is driven by an appropriate promoter (e.g. cytomegalovirus immediate-early (CMV) promoter, or hybrid CAG promoter consisting of the CMV early enhancer and chicken β-actin promoter). Baculovirus cloning capacity is as large as 38 kb (Chessenko et al., 2001), thus allowing for the insertion of multiple genes and regulatory elements (Kost et al., 2005; Kost and Con dreay, 2002). Baculovirus neither replicates inside the transduced cells nor integrates its DNA into host chromosomes in the absence of selective pressure (Chen et al., 2011a; Merrihew et al., 2001), hence easing the safety concerns. Humans do not possess pre-existing antibody and T-cells specifically against baculovirus (Strauss et al., 2007), therefore baculovirus may circumvent the pre-existing immunity problem faced by other viral vectors. Finally, recombinant baculovirus can be readily constructed and propagated to high titers in biosafety level 1 facilities by infecting its natural host insect cells (Hu, 2008). These attributes have fueled growing interests to explore baculovirus for a wide variety of applications, ranging from protein production (Jardín et al., 2008; Liu et al., 2010), virus production (Huang et al., 2007; Lesch et al., 2008, 2011; Lucifora et al., 2008; McCormick et al., 2002; Nakowitsch et al., 2006; Zheng et al., 2010), virus-like particle production (Chen et al., 2005; Matsuho et al., 2006; Wang et al., 2005), eucaryotic protein display (Ernst et al., 2006, Grabherr and Ernst, 2010), vaccine development (Hu et al., 2008; Madhan et al., 2010; Tani et al., 2008), cancer therapy (Wang and Balasundaram, 2010), cell-based assay development (Con dreay et al., 2006; Con dreay and Kost, 2007; Kost et al., 2010) to tissue engineering (Lin et al., 2010b).

This article will focus on recent understandings of intracellular events after baculovirus transduction and latest applications of baculovirus for antiviral therapy, cancer therapy, tissue regeneration and vaccine development.

2. What happens after baculovirus transduction?

2.1. Baculovirus entry and intracellular transport

Baculovirus entry into mammalian cells was initially suggested to depend on electrostatic interactions (Duisit et al., 1999), heparin sulfate (Duisit et al., 1999) and phospholipids (Tani et al., 2001), but the exact cell surface molecules for baculovirus docking remained unknown. It was also proposed that clathrin-mediated endocytosis (Long et al., 2007; Matilainen et al., 2005) and macrophagocytosis (Matilainen et al., 2005) play roles in baculovirus entry. Contradictorily, a recent study (Laakkonen et al., 2009) discovered that (1) baculovirus entered HEK293 and HepG2 cells along fluid-phase markers from the raft areas into vesicles devoid of clathrin; (2) macrophagocytosis-related regulators (e.g. EIPA, Pak1, Rab34 and Rac1) imparted no significant effects on virus transduction and (3) the internalization and nuclear uptake were affected by the regulators of clathrin-independent entry. These data unveiled a baculovirus entry pathway independent of clathrin-mediated endocytosis and macrophagocytosis and suggested

| Table 1 | Selected types of cells permissive to baculovirus transduction. |
|---------|---------------------------------------------------------------|
| Cell type | References |
| Human cells | |
| HepG2 | (Gerner et al., 2010; Laakkonen et al., 2008; Laakkonen et al., 2009) |
| HEK293 | (Efruse et al., 2010; Laakkonen et al., 2009; Lavdas et al., 2010; Sollerbrant et al., 2001) |
| Huh7 | (Lo et al., 2009) |
| Vero E6 cells | (Liu et al., 2009a) |
| Porcine cells | |
| Primary smooth muscle cell | |
| Bone marrow mesenchymal stem cells | |
| Non-human primate cells | |
| CDS-7 | (Pan et al., 2009) |
| CV-1 | (Tani et al., 2001) |
| Vero | (Zheng et al., 2010) |
| Corneal epithelial cell | |
| Antibodies | |
| Rabbit cells | |
| Aortic smooth muscle cell | |
| Intervertebral disk cell | |
| Primary articular chondrocyte | |
| Fish cells | |
| EPC | (Chen et al., 2009c; Lee et al., 2009; Sung et al., 2009) |
| Embryo | (Huang et al., 2011) |
| Fathead minnow muscle | |
| Medaka ES cells | (Yan et al., 2009) |
| Avian cells | |
| DC-1 | (Lu et al., 2007) |
| DT40 | (Huang et al., 2011) |
| MDCK | (Huang et al., 2011) |
| MDCK | (Lu et al., 2007) |
| Feline cells | (Gilbert et al., 2005) |
| Norden Laboratories feline kidney (NFK) | |
baculovirus context, IE2 forms the nuclear foci and develops into large nuclear bodies (NBs) with a hollow center. The IE2 NBs structure contains abundant G-actin, closely associated with RNA polymerase II, promyelocytic leukemia (PML) and small ubiquitin-like modifier-1 (SUMO1) and is the site of active transcription, thereby contributing to the IE2-associated gene stimulation (Liu et al., 2009a). Furthermore, the NBs formation and CMV promoter activation require the N-terminal RING finger and C-terminal coiled-coil domains of IE2 (Liu et al., 2009a). Since CMV promoter is exhaustively used for baculovirus-mediated gene transfer, the transactivating activity of IE2 may be useful for baculovirus-mediated protein production in mammalian cells (Liu et al., 2010).

2.2. Expression of baculoviral genes in permissive cells

Baculovirus encodes ≈ 155 genes and a number of viral genes (e.g. orf149, ie0, p35 and gp64) are expressed in transduced mammalian cells (Fujita et al., 2006; Kitajima et al., 2006). Among the baculoviral genes, ie1 is expressed early in insect cells and transactivates downstream gene expression. Forced expression of ie1 by the minimal CMV promoter in Vero E6 cells also markedly activates gp64 and pe38, and upregulates ie2, he65, pcna, orf16, orf17 and orf25 (Liu et al., 2007). The critical role of ie1 for transactivating downstream genes was further unraveled in a recent study (Erose et al., 2010), which showed that baculovirus deficient in ie1 gene mitigates residual baculoviral gene expression 10- to 100-fold (when compared with wild-type baculovirus) in transduced mammalian cells, thus enhancing safety features to baculovirus-based gene therapy.

In contrast to ie1, ie2 overexpression driven by the CMV promoter only upregulates 2 baculoviral genes (pe38 and orf17), but baculovirus-mediated co-expression of ie1 and ie2 acts in concert to upregulate 59 out of 155 baculovirus genes in mammalian cells (Liu et al., 2007). Strikingly, IE2 is a strong transactivator of CMV promoter in both Vero E6 and U-20S cells (Liu et al., 2008a). When overexpressed within the baculovirus context, IE2 forms the nuclear foci and develops into large

that phagocytosis might play a role (Laakkonen et al., 2009), which echoed the observations reported previously (Abe et al., 2005). Moreover, other recent studies reported that baculovirus transduction related to direct fusion pathway induced by a short pH trigger (Dong et al., 2010; Paul and Prakash, 2010). These conflicting data underlined the need for more in-depth studies to elucidate the underlying mechanism and might suggest that baculovirus entry pathway varies with cell types. Nevertheless, one consensus is that baculovirus envelope protein gp64 is pivotal for entry because blocking gp64 can abrogate the baculovirus ability to transduce mammalian cells (Abe et al., 2005; Niu et al., 2008) and activate dendritic cells (DCs) (Schutz et al., 2006).

Once inside the cells, baculovirus is transported to the endosome, followed by endosomal escape via acid-triggered gp64 fusion (Kukkonen et al., 2003) and subsequent nuclear transport (Laakkonen et al., 2008; van Loo et al., 2001) with the aid of actin filaments (Matilainen et al., 2005; Salminen et al., 2005). A major component of type III intermediate filaments, vimentin, also participates in intracellular trafficking (Mahonen et al., 2010). Vimentin is reorganized in the optimized culture medium and is linked to enhanced nuclear entry of baculovirus, underscoring the importance of culture medium in the cytoskeleton network assembly and in baculoviral gene delivery.

2.3. Innate immune responses elicited by baculovirus

Due to the complex cascade of events during baculovirus transduction, it is not surprising that baculovirus can alter the cell morphology and trigger cellular responses. For instance, baculovirus transduction of HepG2 cells alters the size of PML NBs, induces remodeling of the host cell chromatin (Laakkonen et al., 2008) and arouses extensive ruffle formation on the cell surface (Laakkonen et al., 2009). Shotgun proteomics also attests that baculovirus-transduced HepG2 cells exhibit a slight induction of proteins related to inflammation, cell survival and chromatin function (Gerner et al., 2010).

The most well-characterized baculovirus-induced cellular response is the innate immune response, as manifested by the induction of such cytokines as interferon α/β (IFN-α/β), interleukin-6 (IL-6), IL-8, IL-1β and tumor necrosis factor-α (TNF-α) (Abe and Matsuura, 2010; Tani et al., 2008). Baculovirus transduction of rat chondrocytes also elicits transient expression of IFN-α/β, which attenuates the transgene expression (Lee et al., 2009). Not only the cytokine secretion, in vitro baculovirus transduction also activates human (Schutz et al., 2006) and mouse (Abe et al., 2005) DCs. Moreover, wild-type baculovirus transduction of mouse bone marrow-derived DCs (BMDCs) upregulates the major histocompatibility complex (MHC) I and II and co-stimulation molecules (e.g. CD40, CD80 and CD86) (Suzuki et al., 2010a). The activated BMDCs can further stimulate natural killer (NK) cells upon coculture, as evidenced by the IFN-γ production, CD69 upregulation and cell proliferation.

In contrast to these differentiated, specialized cells, stem cells appear to be less sensitive to baculovirus transduction. Upon wild-type baculovirus transduction, human bone marrow-derived mesenchymal stem cells (BMSCs) secret IL-6 and IL-8, but no detectable levels of IFN-γ, IFN-β, TNF-α, TNF-β, IL-1α, IL-2, IL-4, IL-5, IL-10 and IL-12 (Chen et al., 2009b). Human leukocyte antigen I (HLA-I) is slightly upregulated, but the expression of HLA-II and other surface
markers are barely disturbed (Bak et al., 2010; Chuang et al., 2009). Neither does baculovirus transduction compromise the immunosuppressive properties of BMSCs (Chuang et al., 2009). Conversely, wild-type baculovirus transduction of mouse induced pluripotent stem cells (iPSCs) elicits only the chemokine IP-10, but not other well-known cytokines (unpublished data). BMSCs and iPSCs are promising cell sources for regenerative medicine. The lack of these cytokine responses reduces the risk of mounting strong immune responses after transplantation of baculovirus-transduced BMSCs and iPSCs.

In vivo, baculovirus administration triggers innate immune responses, activates macrophages (Abe et al., 2005), DCs (Hervas-Stubbs et al., 2007; Schutz et al., 2006) and NK cells (Facciabene et al., 2004; Kitajima et al., 2008). The baculovirus–induced innate immunity gives rise to antitumor activity (Suzuki et al., 2010a) and is sufficient to confer protection against influenza virus in mice (Abe et al., 2003), infectious bronchitis virus in chickens (Niu et al., 2009) and foot-and-mouth-disease virus in mice (Molnari et al., 2010). The innate immunity also confers baculovirus the adjuvant activity to promote humoral and cellular immune responses against co-administered antigens (Hervas-Stubbs et al., 2007). Moreover, intramuscular (i.m.) injection of baculovirus triggers T-cell responses against the vector, but the magnitude of anti-baculovirus T cells response is lower than that of anti-adenovirus response (Hervas-Stubbs et al., 2007).

2.4. Baculovirus recognition and associated signaling pathways

Toll-like receptors (TLRs) are a family of pattern recognition receptors essential for initiating the innate immunity and substantiating the adaptive immunity (Ishii et al., 2008). For instance, TLR3 recognizes virus-derived dsRNA while TLR9 recognizes unmethylated CpG DNA motifs. Upon engagement with cognate ligands, TLRs are activated and recruit adaptor molecules such as myeloid differentiating factor 88 (MyD88) and TIR domain-containing adaptor inducing IFN-β (TRIF) to transduce signals to downstream molecules. Besides, stimulator of interferon genes (STING) is a cytoplasmic sensor that activates IRF3 and IFN-α/β in response to viral dsDNA while retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) are TLR-independent cytoplasmic RNA detectors that induce the IFN-α/β production through IFN promoter-stimulator-1 (IPS-1).

Baculovirus–induced innate immunity has been ascribed to the recognition of CpG motifs in viral DNA and ensuing activation of TRIF/MYD88-dependent pathway (Abe et al., 2005). The detection activates nuclear factor–κB (NF-κB) and leads to subsequent IFN-α/β production. However, IFN-α/β are still produced in peritoneal cells derived from MyD88– or TLR9-deficient mice (Abe et al., 2005). In mouse embryonic fibroblasts (MEFs), baculovirus triggers IFN-β and IFN-inducible chemokines through TLR-independent and IRF3-dependent pathways and endosomal maturation is required for induction (Abe et al., 2009). These data suggest that baculovirus DNA might be recognized by at least two different pathways: TLR9-dependent endosomal recognition and TLR9-independent cytoplasmic recognition. The latter was suggested to be related to STING (Abe and Matsuiura, 2010) because baculovirus–induced IFN-α/β production was impaired in STING-deficient MEFs (Ishikawa et al., 2009), yet IFN-α/β stimulation was found to be independent of other cytoplasmic RNA detectors such as RIG-I and MDA5 (Abe et al., 2009). However, a recent study noted that RIG-I and MDA5 mRNA levels were elevated in baculovirus–transduced cells (Wang et al., 2010). Addition of DNA methyltransferase inhibitors (DNMTi) prior to transduction retarded such upregulation and enhanced baculovirus–mediated gene expression, suggesting that DNMTi may somehow facilitate the baculovirus evasion from the cellular recognition and thus ameliorate the transgene expression.

Using the cDNA microarray, Wang and coworkers discovered that baculovirus injection into the striatum in the rat brain perturbed the expression of 628 genes, which represented 3.45% of the total gene probes on the microarray (Boulaire et al., 2009). The same study also identified 532 gene probes (1.99%) in human astrocytes and 1219 gene probes (4.32%) in human neuronal cells that were disturbed in response to baculovirus. Despite the disparity between cell types, in all 3 samples baculovirus altered the expression of genes associated with TLR signaling pathway (e.g. TLR2, TLR3, CCL5, CXCL10 and STAT1) and cytokine–cytokine receptor interaction (e.g. CXCL10, CXCL11 and CCL5). Moreover, genes associated with interferon induction-related genes (e.g. CXCL10, MX1, MX2, OAS1 and STAT1) and antigen processing and presentation pathway (e.g. CD74 and RT-1Ba) were affected. As such, Wang and coworkers proposed that baculovirus recognition by TLRs triggers the expression of IFN-α/β, which initiates the subsequent signaling cascade involving STATs, upregulating the expression of IFN-responsive genes and hence confers the cells the antiviral state. Concurrent with the aforementioned findings, we also discovered that baculovirus transduction of human BMSCs disturbed the expression of IL6 genes, most of which were related to 5 signaling pathways: cell adhesion molecules, TLR, Jak-STAT, apoptosis as well as antigen processing and presentation (Chen et al., 2009b). Of note, baculovirus triggered the TLR3 pathway, resulting in downstream NF-κB and IRF-3 activation and IL-6/IL-8 production. However, how baculovirus containing the dsDNA genome activated the dsRNA sensor TLR3 remains an enigma. Also, the transduction did not arouse the secretion of IFN-β (Chen et al., 2009b), a signature cytokine associated with TLR3 activation, implying a signaling cascade somewhat distinct from that in immune cells.

Interestingly, the budded form of another baculovirus, Antheraea pernyi nuclear polyhedrosis virus (ApNPV), triggers the TLR2 signaling in chicken macrophage-like cells (HD11) but not in chicken B cell-like cells (Han et al., 2010). ApNPV transduction of HD11 cells concomitantly induces the production of IFN-γ, IL-12p40 and nitric oxide (NO) (Niu et al., 2008), which is accompanied by the phosphorylation of extracellular signal-related kinase 1/2 (ERK 1/2), p38 mitogen activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) as well as activation of p65-NF-κB (Han et al., 2009). Inhibition of p38 MAPK and NF-κB by their respective inhibitors abrogates the expression of cytokines and NO, whereas inhibition of JNK abolishes only the induction of cytokines. Since in mammals TLRs signaling activates the downstream NF-κB and MAPK cascade comprised of at least p38 MAPK, ERK 1/2, and JNK (Ishii et al., 2008), these data altogether suggest that ApNPV transduction of HD11 cells activates TLR2 and signals through NF-κB, p38 MAPK and JNK pathways, and chicken TLR21 might play a role similar to mammalian TLR9 (Han et al., 2009, 2010).

3. Modifications of baculovirus for improved transduction

3.1. Surface display via gp64 fusion or heterologous protein

Baculovirus envelope protein gp64 comprises an N-terminal signal peptide and a mature domain that encompasses the transmembrane domain and cytoplasmic domain (CTD). Heterologous protein/peptide has been inserted in between the signal peptide and mature domain, which after expression under the cytomegalovirus immediate early (CMV IE) promoter, is incorporated into the viral envelope upon virus budding. Such feature has been exploited for surface display of protein/peptide to improve the virus transduction (Grabherr and Ernst, 2010; Grabherr et al., 2001; Raty et al., 2004) or for ligand-directed targeting if an appropriate ligand is chosen (Kitagawa et al., 2005; Makela et al., 2006, 2008). For instance, baculovirus poorly transduces B lymphocytes (Cheng et al., 2004; Condreay et al., 1999). Via gp64 fusion, the transduction has been enhanced by displaying the short peptide motif from gp350/220 of Epstein–Barr virus (EBV, which naturally infects B cells) on the baculovirus envelope (Ge et al., 2007). Alternatively, the cytoplasmic transduction peptide (C TP) has been fused to gp64 to
enhance the baculovirus transduction of Vero E6, U-2OS and CHO-RD cells (Chen et al., 2011b). Another paradigm is the display of the fragment crystallisable (Fc) region of antibody on the baculovirus surface (Martyn et al., 2009). Fc receptors (FcRs) are membrane proteins that bind to the Fc region of antibody and mediate the phagocytosis and antigen presentation. The Fc display allows for specific baculovirus targeting to cell lines and antigen presenting cells (APCs) expressing FcRs, hence augmenting the vaccine effect (Martyn et al., 2009). The display system also allows for the surface presentation of functional membrane proteins to simplify subsequent isolation (Zhang et al., 2008).

Aside from the gp64-aided display, expression of vesicular stomatitis virus G protein (VSVG) (Chapple and Jones, 2002; Makela and Oker-Blom, 2006), influenza virus neuraminidase (Borg et al., 2004), Spodoptera exigua multiple nucleopolyhedrovirus F protein (Yu et al., 2009), single chain antibody fragments (Kidiebe et al., 2010) and human endogenous retrovirus envelope protein (Lee et al., 2010) in insect cells also leads to incorporation of the protein into baculovirus envelope. Among these strategies, display of VSVG or heterologous peptide/protein via the VSVG anchor is the most widely adopted and can tremendously enhance baculovirus transduction in vitro and in vivo (Facciabene et al., 2004; Kaikkonen et al., 2006; Kaneko et al., 2006; Kitagawa et al., 2005; Lu et al., 2006; Tani et al., 2003, 2001; Zhou and Blissard, 2008).

Serum complement components (e.g. C5b-9) inactivate baculovirus, hence constituting a major hurdle in the in vivo use of baculovirus. The inactivation problem has been circumvented by the use of complement inhibitors such as compstatin (Georgopoulos et al., 2005) and soluble complement inhibitor 1 (Hoare et al., 2005; Hofmann et al., 2009), avoiding exposure to the complement (Airenne et al., 2000) or administering the vector to immunoprivileged sites (Kinnunen et al., 2004; Lehtolainen et al., 2002). Alternatively, the baculoviral vector can become complement-resistant by displaying human DAF (decay accelerating factor) via gp64 fusion (Huser et al., 2001, Kaname et al., 2001). More recently, baculoviral vectors displaying different complement regulatory proteins (e.g. DAF, factor H-like protein-1, C4b-binding protein and membrane cofactor protein) were generated by fusion with the membrane anchor of VSVG (Kaikkonen et al., 2010). These surface-modified vectors exhibited varying degrees of complement resistance in vitro and DAF yielded the highest level of protection. Intraperitoneal delivery of the DAF-displaying baculovirus increased the survival and gene expression in immunocompetent mice. The DAF-displaying baculovirus provoked lower levels of inflammatory cytokines IL-1β, IL-6, and IL-12p40 in macrophages and mitigated liver inflammation in mice when compared with the control virus. These results prove that DAF display offers protection to the baculoviral vector against complement inactivation and attenuates complement-mediated inflammation injury.

3.2. Surface modification via capsid display, chemical coupling or electrostatic interactions

Other than the display on the envelope, heterologous protein has been displayed on the capsid by fusion with the major capsid protein VP39. The VP39 fusion with enhanced green fluorescent protein (EGFP) neither interferes with the virus assembly nor affects the virus tier, thereby enabling intracellular baculovirus trafficking and biodistribution monitoring (Kukkonen et al., 2003). Similarly, the ZnO binding peptide has been fused to the N-terminus of VP39 while retaining the viral infectivity and conferring the ability to bind nanosized ZnO powders (Song et al., 2010). Besides, by fusing the protein transduction domain (PTD) of human immunodeficiency virus (HIV) TAT protein (a protein responsible for nuclear import of HIV genome) with VP39, the engineered baculovirus results in improved transduction of various mammalian cells (Chen et al., 2011b).

In addition to surface modification by genetic engineering, baculovirus has been labeled with tracers for tracking (Li et al., 2004) or biodistribution imaging (Raty et al., 2006; Raty et al., 2007). Chemical coupling of antigenic peptides also enables rapid modification of baculovirus particles and delivery of multiple epitopes (Wilson et al., 2008). Baculovirus can also be chemically conjugated with polyethylene glycol (PEG) alone (Kim et al., 2006) or together with folate (Kim et al., 2007, 2010) to improve the transduction of folate receptor-positive KB cells. Additionally, baculoviral vectors have been coated with positively charged polyethylenimine (25 kDa) through electrostactic interactions (Yang et al., 2009). The modification imparts baculoviral vectors resistance to human and rat serum-mediated inactivation in vitro and elevates in vivo transduction in the liver and spleen after tail vein injection into mice.

4. Baculovirus as an RNA interference (RNAi) mediator

RNA interference (RNAi) is a phenomenon that mediates sequence-specific post-transcriptional gene silencing and can be artificially elicited by the expression of short hairpin RNAs (shRNA) or microRNA (miRNA) precursors from an expression vector (Garzon et al., 2010). Since the initial demonstrations of baculovirus-mediated shRNA delivery (Nicholson et al., 2005; Ong et al., 2005), baculovirus-mediated RNAi has been explored for antiviral therapy. One baculovirus expressing the shRNA specific for the C-terminal nucleoprotein of porcine reproductive and respiratory syndrome virus (PRRSV) genome inhibits the viral replication in vitro (Lu et al., 2006). The baculovirus harboring a bispecific shRNA targeted against influenza virus A and B can inhibit the production of both virus types in transduced cell lines (Suzuki et al., 2009b). Another baculovirus expressing shRNA against the peste des petits ruminants virus (PPRV) represses PPRV replication in vitro and the inhibition effect is superior to that mediated by the adenosivirus expressing the same shRNA (Nizamani et al., 2011). Hepatitis B virus (HBV) covalently closed circular (CCC) DNA is the source of HBV transcripts in chronically infected patients. Baculovirus expressing the HBV-specific shRNA is able to hinder the formation of HBV CCC DNA (Starkey et al., 2009). Additionally, the baculovirus expressing shRNAs specific for the highly conserved core region of hepatitis C virus (HCV) genome dramatically impedes the target protein expression (Suzuki et al., 2009c).

The replication and segregation of EBV genomes to daughter cells is coordinated by the binding of EBV nuclear antigen 1 (EBNA1) to oriP, an origin of replication derived from EBV. To prolong the transgene expression, baculoviral vectors incorporating the oriP/EBNA1 sequences were developed (Shan et al., 2006; Wang et al., 2008). Based on this concept, Suzuki et al. devised a baculoviral vector that accommodated the oriP/EBNA1 sequence and encoded the HCV-specific shRNA. This vector inhibited HCV core protein expression for >14 days, which considerably outlasted the 3 days of inhibition conferred by the conventional vector (Suzuki et al., 2009a).

Recently, we also exploited the baculovirus vector for miRNA delivery and gene regulation. The baculovirus vectors carried artificial gene-specific miRNA sequences within the mir155 scaffold, which after expression under the CMV promoter could undergo the miRNA processing pathway and knocked down the target gene (e.g. EGFP and TNF-α) expression (unpublished data). The gene suppression effect was extended by flanking the miRNA expression cassette with the inverted terminal repeat sequences/direct repeats sequences (IR/DR) recognized by the Sleeping Beauty (SB) transposase. Co-transduction of cells with the hybrid baculovirus/transposon vector and another SB transposase-expressing baculovirus resulted in the integration of miRNA expression cassette into the chromosome, giving rise to prolonged target gene suppression (unpublished data). These data altogether implicate the potential of baculovirus-based RNAi shuttle technology.
for antiviral therapy and treatment of indications necessitating prolonged gene regulation (e.g. arthritis).

5. Baculovirus as a vector for cancer gene therapy

5.1. Direct baculovirus injection for cancer therapy

The feasibility of baculovirus-mediated cancer therapy was first explored by Wang et al. (2006), who developed a baculoviral vector expressing the Diphtheria Toxin A and demonstrated that the baculovirus impeded the growth of cultured glioma cells and glioma xenograft in the rat brain. Since then, baculovirus has been utilized for the treatment of mouse hepatoma (Kitajima et al., 2008), murine melanoma, lung cancer and brain cancer (Kim et al., 2007). Recently, Wang and coworkers constructed another baculovirus that selectively completely eradicated after one single baculovirus injection, presumably prolonged the mouse survival. However, the tumor mass was not in normal brain tissues. This vector triggered death of glioblastoma cells in the presence of ganciclovir, but did not affect the survival of human astrocytes and neurons. In a mouse xenograft model, intratumoral injection of this baculovirus at 7 days after tumor inoculation suppressed the growth of human glioblastoma and prolonged the mouse survival. However, the tumor mass was not completely eradicated after one single baculovirus injection, presumably due to the transient HSVtk expression (Balani et al., 2009). Alternatively, the HSVtk gene was driven by the GFAP (glial fibrillary acidic protein) promoter whose activity is similar in normal and tumor cells of the same lineage (Wu et al., 2009a). To limit the transgene expression in glioma cells, repeated target sequences of 3 miRNAs (has-miR31, has-miR127 and has-miR143) that are enriched in astrocytes but are sparse in glioblastoma cells were appended to the 3\(^{′}\) untranslated region of the HSVtk gene. The baculovirus vector markedly improved in vivo selectivity when compared with the control vector without miRNA regulation, effectively inhibited human glioma xenograft and imparted negligible toxicity to normal astrocytes. The incorporation of selected miRNA sponge thus provides an additional safety switch to prevent off-target transgene expression (Wu et al., 2009a).

In addition to HSVtk, the baculovirus expressing NES1 (normal epithelial cell specific-1) can inhibit the growth of gastric cancer cells (SGC-7901) in vitro and repress the SGC-7901 xenografted tumor growth after intratumoral injection, implicating the possibility for the therapy of gastric and colon cancers (Huang et al., 2008). Baculovirus can also carry tumor suppressor genes such as p53 or pdc4d (programmed cell death 4) for cancer treatment. In the mouse glioma xenograft models, the antitumor effect of the baculovirus-expressed p53 was substantiated by surface coating of the baculovirus envelope with polyethyleneimine (Yang et al., 2009) or by co-administering sodium butyrate (Guo et al., 2011), a histone deacetylase inhibitor that ameliorates baculovirus-mediated gene expression (Guo et al., 2010; Hu et al., 2003; Yin et al., 2010; Zhou et al., 2010). The PDCD4-expressing baculovirus conjugated with folate-PEG efficiently expressed PDCD4 protein and induced apoptosis in human epidermal carcinoma cells (Kim et al., 2010). In a tumor xenograft, intratumoral injection of the PDCD4-expressing baculovirus significantly suppressed tumor growth and induced apoptosis. Moreover, apoptin is a chicken anemia virus-derived protein that specifically triggers apoptosis in tumors. The VSVG-pseudotyped baculovirus expressing apoptin efficiently provoked apoptosis in mammalian cells, repressed the growth of xenograft tumors and prolonged the mice survival after intratumoral injection (Pan et al., 2010).

One determinant to the baculovirus-mediated antitumor effects is the innate immune responses elicited by the virus (Suzuki et al., 2010b). Takaku and coworkers demonstrated that wild-type baculovirus, after intravenous (i.v.) injection into immunocompetent B6 mouse, was taken up by the liver and spleen, preferentially entered DCs and B cells, activated DCs, induced NK cells proliferation in the liver and spleen, and enhanced the antitumor immunity in mice with B16 liver metastases (Kitajima et al., 2008). Baculovirus administration also increased the survival of C57BL/6, Jx281\(^{−/−}\) and IFN-γ\(^{−/−}\) mice bearing the B16 tumor, but did not enhance the survival of NK cell-depleted mice. These results prove that wild-type baculovirus efficiently induces NK cell-mediated antitumor immunity (Kitajima et al., 2008).

5.2. Baculovirus in conjunction with cell therapy for cancer treatment

Besides direct injection, baculovirus has been employed in conjunction with cell therapy for cancer treatment. Bone marrow-derived DCs (BMDCs), after ex vivo transduction with wild-type baculovirus and i.v. injection into mice, significantly suppressed the lung cancer (Suzuki et al., 2010a). In a mouse melanoma model, baculovirus-transduced BMDCs inhibited tumor growth and improved animal survival at least partly due to the induction of CD8+ T cell- and NK cell-dependent, CD4+ T cell-independent antitumor immunity. Importantly, BMDCs administration did not provoke evident signs of damage to the liver or kidney, as judged from the negligible disturbance of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine levels (Suzuki et al., 2010a). Another promising cell courier is BMSCs thanks to their intrinsic tumor homing property. In this regard, BMSCs were transduced with a baculovirus expressing HSVtk and injected via tail vein into the mice pre-inoculated with human U87 glioma cells (Bak et al., 2010). After ganciclovir injection, tumor growth was significantly repressed and the life span of animals was considerably prolonged. More recently, the same group generated MSC-like cells from human embryonic stem (ES) cells which, after transduction with the baculovirus expressing HSVtk and injection into the brain, were capable of inhibiting tumor growth and prolonging the survival of tumor-bearing mice in the presence of ganciclovir (Bak et al., 2011). These data implicated the feasibility of human ES cells-derived MSCs as a viable and attractive alternative for cancer therapy.

6. Baculoviral vector for regenerative medicine

Gene therapy has converged with tissue engineering, by which the therapeutic genes stimulating tissue regeneration can be administered to cells either in vivo or ex vivo with an appropriate vector. Given the highly efficient gene delivery (Lin et al., 2010b).

6.1. Baculovirus-mediated cartilage regeneration

Articular cartilage is a weight-bearing tissue comprising chondrocytes and extracellular matrix (ECM) composed of proteins (e.g. collagen II) and glycosaminoglycans (GAGs) such as aggrecan. Articular cartilage may be damaged due to direct trauma or joint diseases, but its ability to self-repair is limited, ultimately leading to debilitating pain and physical impairment. Therefore, cartilage tissue engineering that combines cells, scaffolds and biological signals has emerged for cartilage repair.

Given that chondrocytes are pivotal in the synthesis, composition modulation, structural arrangement of ECM components and hence the mechanical properties, we developed a protocol for baculovirus transduction of rat (Ho et al., 2004) and rabbit (Sung et al., 2007) chondrocytes. This protocol involves the incubation of cells with the virus at 25–27 °C for 4–8 h using Dulbecco’s phosphate-buffered saline (D-PBS) as the surrounding solution and confers efficiencies higher than 90% (Chen et al., 2006). The key to such high transduction efficiencies is the absence of NaHCO\(_3\) in D-PBS, which hinders the
baculovirus transduction (Shen et al., 2007). Consequently, the surrounding solution has been replaced with NaHCO₃-deficient DMEM medium (Lo et al., 2009). Critically, chondrocytes transduced with an EGFP-expressing baculovirus remain capable of producing cartilage-specific collagen II and GAGs, and grow into cartilaginous tissues when seeded into the porous, poly-(l-lactide-co-glycolide) (PLGA) scaffold and cultivated dynamically (Chen et al., 2006). These data demonstrate that baculovirus transduction of chondrocytes does not obstruct the chondrocytes differentiation. Therefore, we further constructed 3 baculovirus vectors each expressing one growth factor (TGF-β1, insulin-like growth factor-1 (IGF-1) or bone morphogenetic protein-2 (BMP-2)) known to stimulate chondrogenesis, and confirmed the protein expression in chondrocytes isolated from New Zealand White (NZW) rabbits (Sung et al., 2007). Among the 3 baculovirus vectors, only the BMP-2-expressing baculovirus (designated Bac-CB) remarkably enhanced the aggrecan and collagen II production by partially de-differentiated passage 3 (P3) cells and restored the differentiation. The baculovirus expressing TGF-β1 (designated Bac-CT) modestly augmented the chondrogenesis but was insufficient to reverse the de-differentiation status of P3 cells (Sung et al., 2007). Nonetheless, co-transduction of de-differentiated P3 chondrocytes with Bac-CB and Bac-CT synergistically modulated the re-differentiation program (Sung et al., 2009).

Albeit the chondrogenic potential, Bac-CB transduction alone was insufficient to support uniform 3D cartilage growth in the static culture due to the lack of mechanical stimulation and poor oxygen/nutrient transfer. To tackle these problems, P3 chondrocytes transduced with Bac-CB were seeded into PLGA scaffolds and cultured in a rotating-shaft bioreactor (RSB), which grew into cartilage-like tissues after 3-week dynamic culture (Chen et al., 2008). Implantation of the engineered cartilages into the osteochondral defects of NZW rabbits accelerated the healing, improved the bone quality and angiogenesis when compared with transplanting ASCs engineered by the conventional baculovirus (Lin et al., 2011). Allotransplantation of the NZW rabbit ASCs transduced with the hybrid baculoviruses expressing BMP-2/VEGF into the critical-size femoral segmental defects accelerated the healing, improved the bone quality and angiogenesis when compared with transplanting ASCs engineered with the conventional baculoviruses (Fig. 2). Therefore, ASCs engineered by the hybrid baculovirus vector hold promise for repairing massive segmental defects (Lin et al., 2011).

The safety profile of the hybrid baculovirus vectors was recently assessed using human BMSCs as the host (Chen et al., 2011a). Transduction of human BMSCs with the hybrid baculovirus neither compromised the cell viability/differentiation, nor resulted in transgene integration into the host chromosome. The transduction did not disrupt the BMSCs karyotype, nor did it disturb the expression of well-known proto-oncogenes (c-myc, N-ras, K-ras and H-ras) and tumor suppressor genes (p53 and p16). Furthermore, the transduced BMSCs did not induce tumor formation in nude mice. These data altogether ensure the safe use of the FLP/Frt-based hybrid vector for stem cell engineering and tissue regeneration.

7. Baculoviruses as vaccine vectors

As discussed in Section 2.3, wild-type baculovirus triggers the innate immunity and potentiates the adaptive immune responses, which protect the animals from the infection of several viruses. These attributes have sparked explosive interests to develop baculovirus as a vector vaccine candidate, in which the antigens can be (1) expressed by the vector within the host cells, (2) displayed on the baculovirus surface or (3) displayed and expressed by the vector (Table 2).

7.1. Baculovirus as an antigen expression vector

The feasibility of using baculovirus as a vaccine expression vector was first tested by in vivo inoculation of the baculovirus expressing the glycoprotein gB of pseudorabies virus (Aoki et al., 1999) or hemagglutinin (HA) of H1N1 influenza virus (Abe et al., 2003) into mice, which raised antigen-specific antibody. Moreover, baculovirus expressing the E2 glycoprotein of HCV or carcinoembryonic antigen
could elicit antigen-specific T cell responses (Facciabene et al., 2004). Similarly, subcutaneous (s.c.) and intraperitoneal (i.p.) immunizations of mice with a baculovirus expressing the antigens of severe acute respiratory syndrome coronavirus (SARS-CoV) induced humoral immune responses and Th1-biased cellular immunity (Bai et al., 2008).

The efficacy of baculovirus-based vaccines has been potentiated by surface display of VSVG protein on the envelope. The VSVG-pseudotyped baculovirus expressing the GP5 and M proteins of porcine reproductive and respiratory syndrome virus (PRRSV) under the CMV promoter elicited anti-PRRSV neutralizing antibodies and IFN-γ, and conferred better immunogenicity than the DNA vaccine expressing the same antigens (Wang et al., 2007). Conversely, i.m. immunizations of a mixture of VSVG-pseudotyped baculovirus expressing pseudorabies virus (PRV) proteins triggered Th1-biased immune responses, as manifested by the induction of IFN-γ and PRV-specific IgG2a antibodies (Grabowska et al., 2009). The immunization also provoked NK cell activity (accompanied by the production of IFN-α and IFN-γ) and protected mice against lethal PRV challenge. I.m. immunization of chickens with another VSVG-pseudotyped baculovirus expressing HA of H5N1 avian influenza virus (AIV) also evoked significantly higher levels of H5-specific antibody and cellular immunity than those receiving DNA vaccines, and conferred protection against lethal challenge with the homologous virus strain (Wu et al., 2009b). Similar VSVG-pseudotyped baculovirus vectors have been constructed to express the Japanese encephalitis virus (JEV) envelope protein (Li et al., 2009b), the capsid protein of porcine circovirus type 2 (PCV2) (Fan et al., 2008), and the Toxoplasma gondii SAG1 protein (Fang et al., 2010). All these studies showed that i.m. immunization of the VSVG-pseudotyped baculovirus vector results in stronger vaccine effects than the DNA vaccine counterparts, which can be attributable to the adjuvant properties of baculovirus and more efficient transduction of muscle cells in vivo. The effective transduction of muscle cells is desired as the chance of antigen presentation to DCs is increased and baculovirus-mediated expression is considerably prolonged in myogenic cells (Shen et al., 2008). Note, however, that VSVG pseudotyping induces cytotoxicity and impairs the baculovirus titer, hence a truncated version of VSVG comprised of the 21 amino acid ectodomain, the transmembrane and CTD domains was designed for baculovirus pseudotyping (Kaikkonen et al., 2006). The truncated VSVG reduced the cytotoxicity and was exploited to construct two pseudotyped baculoviruses: one expressing the capsid protein of foot-and-mouth disease virus (FMDV) and the other encoding the capsid and a T-cell immunogen (Cao et al., 2011). I.m. inoculation of the

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**Fig. 2.** The ASCs engineered with the hybrid baculovirus augment the healing of massive bone defects. The NZW rabbit ASCs were transduced with the hybrid baculovirus vectors conferring sustained expression of BMP-2 or VEGF, mixed at a number ratio of 4:1, loaded into cylindrical PLGA scaffolds (1.5 × 10⁶ cells/scaffold) and implanted to the critical-size segmental defects at the femora of NZW rabbits (2 constructs/defect, designated L group). The S group contained ASCs that were transduced with conventional baculoviruses transiently expressing BMP-2/VEGF and implanted in a similar fashion. The Mock group comprised the mock-transduced ASCs as the negative control. X-ray radiography, gross appearance examination, micro computed tomography (μCT), hematoxylin and eosin (H&E) staining and CD 31-specific immunohistochemical staining (to detect blood vessel formation) performed at 12 weeks post-implantation collectively demonstrated that the L group (persistently expressing BMP-2 and VEGF) resulted in significantly improved bone healing and angiogenesis in comparison with the S group (transiently expressing BMP-2 and VEGF) and Mock group. Stars indicate the new bone while arrows indicate the blood vessel formation.
baculoviruses into mice induced the FMDV-specific neutralizing antibodies and IFN-γ, and expression of the additional T-cell immunogen augmented the immunogenicity.

Besides VSVG, human endogenous retrovirus (HERV) envelope protein was used for pseudotyping the baculovirus (designated AcHERV-HP16L1) that encoded the L1 capsid protein of human papillomavirus 16 (HPV 16) (Lee et al., 2010). After i.m. injection and 2 booster injections at 2-week intervals, the mice developed similarly high levels of IgG, IgA and neutralization titers when compared with the mice immunized with the commercial virus-like particle vaccine Gardasil® (25 μL/dose). Thus AcHERV-HP16L1 holds promise as a cost-effective efficient HPV vaccine.

7.2. Baculovirus as an antigen display vector

As mentioned in Section 3.1, surface display of heterologous protein/peptide has been achieved by fusion with gp64 gene and expression driven by either polyhedrin or p10 promoter. Taking advantage of this technology, baculovirus displaying the rodent malaria Plasmodium berghei circumsporozoite protein (PbCSP) (Yoshida et al., 2003), the antigenic site A of FMDV VP1 protein (Tami et al., 2004) or the SARS-CoV spike protein (Chang et al., 2004; Feng et al., 2006) has been shown to induce potent immune responses. More recently, baculovirus vectors displaying the Pf525 protein of Plasmodium falciparum (Mlambo et al., 2010) and 19-kDa carboxyl terminus of merozoite surface protein 1 (PfEMP1) (Yang et al., 2007) have also been developed as potential vaccines against malaria. I.m. and intranasal (i.n) immunizations with the PfEMP1-displaying baculovirus induced mixed Th1/Th2-type immune responses, but i.n. immunization yielded higher PfEMP1-specific antibody titers and natural boosting after challenge. I.n. immunization also conferred complete protection thanks to the Th1/Th2-type immunity associated with TLR9-dependent pathway (Yoshida et al., 2010).

HA protein is the major antigen of influenza virus and, similar to gp64, a class I transmembrane protein on the viral envelope. gp64-based fusion was harnessed to display the HA of AIV using the CTD derived from HA or from gp64 (Yang et al., 2007). In comparison with the HA CTD, gp64 CTD endowed more efficient HA display and stimulated stronger hemagglutination inhibition (HI) titers crucial for neutralizing the live AIV (Yang et al., 2007). The profound effect of gp64 CTD on HA display was attested in a recent study which delineated that the signal peptide and CTD of gp64 could enhance the display of influenza HA on baculovirus surface, while the gp64 transmembrane domain impaired HA display (Tang et al., 2010). Based on the finding, a baculovirus simultaneously displaying 4 HAs derived from 4 subclades of H5N1 AIV was constructed. I.m. immunization of mice with this tetravalent baculovirus elicited HI titers against all 4 homologous H5N1 viruses, significantly reduced viral lung titers of challenged mice, raised high levels of IFN-γ-secreting and HA-specific CD8+ T cells, and provided 100% protection against lethal challenge with homologous H5N1 viruses (Tang et al., 2010). The same design concept exploiting the gp64 CTD was also adopted to display the εC and εB proteins of avian reovirus (Lin et al., 2008), Eδ envelope glycoprotein (Xu et al., 2008), E2 (Xu and Liu, 2008) or NS3 protein (Xu et al., 2009) of classical swine fever virus (CSFV) and the E glycoprotein of JEV (Xu et al., 2011). These studies collectively attested that the antigen can be efficiently displayed on the baculoviral envelope and induce humoral/cellular immune responses against the lethal viral challenge.

Other than displaying the antigen via genetic engineering, CD4+ T helper epitope (SFERFEEIPKE) and the major B cell epitope (WLTEKEGSYP) derived from the HA of influenza virus (Wilson et al., 2008) have also been chemically conjugated to baculovirus envelope (Wilson et al., 2008). I.n. administration of such baculovirus to mice elicited antigen-specific IgG1/2a, IgA and IFN-γ, thus chemical coupling allows for the delivery of multiple epitopes to baculovirus. In addition, HA of AIV (H5N1) has been displayed on another baculovirus Bombyx mori NPV (BmNPV) via fusion with gp64 (Jin et al., 2008). The virus was produced and purified from silkworm pupae infected with the recombinant virus. Immunization of rhesus monkeys with aluminum hydroxide as the adjuvant at doses of 2 mg/kg and 0.67 mg/kg elicited neutralizing antibodies and protected monkeys against influenza virus challenge. The vaccine did not cause appreciable toxicity at the dose as large as 3.2 mg/kg in cynomolgus monkeys and 1.6 mg/kg in mice, indicating the safe vaccine doses (Jin et al., 2008).

| Strategies | Antigens | Pathogens | References |
|-----------|----------|-----------|------------|
| Expression | Hemagglutinin protein | Influenza virus | (Wu et al., 2009b) |
| E envelope glycoprotein | Japanese encephalitis virus | (Li et al., 2009b) |
| gB, gC, and gD glycoproteins | Pseudorabies virus | (Grabowska et al., 2009) |
| Spike and nucleocapsid proteins | SARS-like coronavirus | (Bai et al., 2008) |
| Capsid protein | Porcine circovirus type 2 | (Fan et al., 2008) |
| Capsid protein | Food-and-mouth disease virus | (Cao et al., 2011) |
| GP5 and M proteins | Porcine reproductive and respiratory syndrome virus | (Wang et al., 2007) |
| HPV-16L1 protein | Human papillomavirus | (Lee et al., 2010) |
| SAG1 protein | Taqspasma gondii | (Fang et al., 2010) |
| Display | Hemagglutinin protein | Influenza virus | (Jin et al., 2008; Tang et al., 2010; Yang et al., 2007) |
| Peptides of hemagglutinin protein | Influenza virus | (Wilson et al., 2008) |
| E envelope glycoprotein | Japanese encephalitis virus | (Xu et al., 2011) |
| Glycoprotein D | Bovine herpesvirus-1 | (Peralta et al., 2007) |
| εB and εC proteins | Avian reovirus | (Lin et al., 2008) |
| Eδ, E2, and NS3 proteins | Classical swine fever virus | (Xu et al., 2008; Xu et al., 2009; Xu and Liu, 2008) |
| 19-kDa carboxyl terminus of merozoite surface protein 1 | Plasmodium yoelii | (Yoshida et al., 2010) |
| Pf525 protein | Plasmodium falciparum | (Mlambo et al., 2010) |
| Dual | Hemagglutinin protein | Influenza virus | (Chen et al., 2010; He et al., 2008; Lu et al., 2007; Prabakaran et al., 2010a, 2010b, 2010c) |
| VP28 protein | White spot syndrome virus | (Syed Mustaq et al., 2009) |
| E2 protein | Classical swine fever virus | (Li et al., 2009a) |
| Psv25 protein | Plasmodium vivax | (Blagborough et al., 2010) |
| Circumsporozoite protein (PbCSP) | Plasmodium berghei | (Yoshida et al., 2009) |
| Circumsporozoite protein | Plasmodium falciparum | (Strauss et al., 2007) |
7.3. Baculovirus as a dual vector for antigen expression/display

It is perceivable that the antigen-expressing baculovirus mimicks the DNA vaccine. Following vector injection into the hosts, the antigen is expressed and presented via MHC I pathway in the transduced cells, and activates CD8+ T cells. Conversely, the antigen-displaying baculovirus mimicks the subunit vaccine. Antigens on the envelope are internalized by professional antigen presenting cells (APCs) such as macrophages and DCs, and presented by the MHC II pathway to stimulate humoral immune responses.

To fully exploit both mechanisms, a dual baculovirus vector that simultaneously displays and expresses the Plasmodium falciparum circumsporozoite (CS) protein was developed as a human malaria vaccine (Strauss et al., 2007). This vector contained two expression cassettes, one encoding the CS-gp64 fusion protein under the polyhedrin promoter while the other accommodating the CS gene under the CMV promoter, such that CS can be displayed on the envelope and expressed after transduction into mammalian cells. Upon i.m. injection into mice, the dual vector induced higher anti-CS antibody titers and higher frequencies of CS-specific CD4+ and CD8+ T cells than the vectors that only displayed or only expressed CS.

Recently, we adopted a similar approach and constructed 3 vectors: Bac-HA64 harbored the HA gene of AIV under the p10 promoter for HA display; Bac-CHA expressed HA under the CMV promoter while the dual vector Bac-CHA-HA64 encompassed both expression cassettes in opposite orientations, such that Bac-CHA/HA64 displayed and expressed HA in the transduced cells (Chen et al., 2010). All 3 vectors, after administration (i.m., i.n. and s.c.) into BALB/c mice, provoked HA-specific humoral (IgG1, IgG2a and HI titers), mucosal (IgA titers) and cellular (IFN-γ and IL-4 producing T cells and IFN-γ/IL-4+ T cells) immune responses. Strong cellular immunity provoked by Bac-HA64, which in theory favors the MHC II pathway and preferentially elicits humoral immune responses, was likely due to the potent adjuvant effects of baculovirus. Regardless, via either administration route the dual vector Bac-CHA-HA64 triggered superior or at least comparable HA-specific immune responses than the other two vaccine forms, demonstrating the advantages of the dual form for vaccine design (Chen et al., 2010).

Instead of using two separate cassettes as above, Yoshida et al. (2009) devised a single cassette system whereby the PbCS–gp64 fusion gene was driven by a tandem promoter consisting of the CMV and polyhedrin promoters, so that PbCS was expressed in insect cells and mammalian cells. i.m. immunization with this baculovirus elicited both Th1 and Th2 responses as evidenced by the high PbCS-specific IgG1/ IgG2a titers and PbCS-specific CD8+ T cells responses, and conferred 100% protection against sporozoite challenge (Yoshida et al. 2009). The same dual expression system was subsequently utilized to express Plasmodium vivax transmission-blocking immunogen (Pvs25) for the development of malarial transmission-blocking vaccines against the sexual stages of the parasite (Blagborough et al., 2010). Both i.m. and i.n. immunizations of mice induced a mixed Th1/Th2 response as evidenced by high Pvs25-specific IgG1 and IgG2a/IgG2b titers as well as a strong transmission-blocking response after challenge.

Another simple approach to developing the dual vector is to drive the antigen expression using the white spot syndrome virus (WSSV) ie1 promoter, which is active in insect, mammalian and avian cells (Gao et al., 2007; He et al., 2008). Baculovirus encoding the WSSV VP28 envelope protein driven by the WSSV ie1 promoter was able to display VP28 on the envelope (Syed Musthaq et al., 2009). Immunization of shrimp with this baculovirus resulted in VP28 expression in shrimp tissues, elevated the survival rate and reduced the WSSV viral load after WSSV infection. WSSV ie1 promoter was also used in conjunction with VSVG pseudotyping. i.m. injection of the VSVG-pseudotyped baculovirus that expressed the E2 protein of CSFV under the ie1 promoter induced CSFV-specific neutralizing antibodies and lymphoproliferative responses in mice (Li et al., 2009a).

WSSV ie1 promoter has been most extensively employed by the Kwang group to drive the expression of HA of H5N1 AIV, which enabled HA display on the envelope and conferred the viral particle hemagglutination activity (Lu et al., 2007). i.m. injection of this vector (designated BacHA) into mice elicited the antibodies with HI titers against the tested influenza virus. In SF-9 cells, WSSV ie1 promoter was more active than CMV promoter, thereby giving rise to more efficient HA incorporation into the baculoviral envelope (He et al., 2008). The ie1 promoter also resulted in strong HA expression in the lung (after i.n. inoculation) and thymus (after i.m. inoculation) in chickens. As a result, immunization of chickens with the baculovirus bearing the ie1 promoter (BacHA) elicited higher anti-HA antibody levels than that bearing the CMV promoter (He et al., 2008). However, i.n. inoculation of BacHA stimulated low anti-HA IgG titers (Prabakaran et al., 2008). To enhance the vaccine efficacy via the i.n. route, BacHA was co-administered with recombinant cholera toxin B subunit (rCTB) as the adjuvant, which significantly enhanced the serum IgG, mucosal IgA and serum microneutralization titers. With the adjuvant, BacHA also triggered higher HA-specific humoral and mucosal immune responses than the inactivated H5N1 virus adjuvanted with the same dose of rCTB, and conferred complete protection against challenge with homologous and heterologous H5N1 strains (Prabakaran et al., 2008).

Additionally, gastrointestinal delivery of BacHA into mice by oral gavage led to transduction in vivo and remarkably boosted the HA-specific IgG, HI and mucosal IgA titers (Prabakaran et al., 2010c). The live BacHA triggered stronger cross-clade neutralization against heterologous H5N1 strains than the inactivated BacHA, and provided 100% protection against challenge with lethal doses of homologous and heterologous H5N1. Moreover, after challenge the immunized mice exhibited only minimal bronchitis in lungs and regained their body weight more rapidly (Prabakaran et al., 2010c).

The oral vaccine efficacy was further potentiated by encapsulating the live BacHA within a reverse micelle structure of phosphatidylcholine, which provided protection against the destructive environment in the intestinal lumen (Prabakaran et al., 2010b). In comparison with the non-encapsulated BacHA, gastrointestinal delivery of the encapsulated baculovirus into mice led to significantly ameliorated HA-specific IgG and IgA responses, and higher HI titers. The encapsulated vaccine induced strong cross-clade neutralization titers against heterologous H5N1 strains and conferred protection against infection with highly pathogenic, heterologous H5N1 viruses (Prabakaran et al., 2010b).

Using the same WSSV ie1 approach, more recently Kwang and coworkers selected 3 HA proteins from different vaccine strains for expression and display on the baculovirus surface (Prabakaran et al., 2010a). The 3 HA proteins covered the entire variants in the neutralizing epitopes among the H5N1 lineages. S.c. immunization of mice with a mixture of 3 baculoviruses displaying the 3 HA proteins (tri-BacHA) induced antibodies capable of neutralizing viruses from clades 1, 2.1, 2.2, 4, 7, and 8 of H5N1 viruses. In contrast, s.c. immunization with a single HA-displaying baculovirus (mono-BacHA) or a single strain of inactivated whole virus vaccine neutralized only clade 1 (homologous), clade 2.1, and clade 8.0 viruses. Also, the tri-BacHA vaccine protected 100% of the mice against challenge with three different clades (clades 1.0, 2.1, and 7.0) of H5N1 viruses.

8. Conclusions

Since the discovery in 1995 that baculovirus effectively transduces mammalian cells and mediates transgene expression, baculovirus has emerged as a promising gene delivery vector. Albeit the rapidly growing lists of permissible cells and applications, relatively little is known about what happens inside the cells after baculovirus transduction. Evidence accumulating in recent years has indicated that after entry, baculovirus can translocate into the nucleus through a...
complex cascade of steps and express baculoviral genes and the transgene. The entire process results in the recognition of baculovirus by the cells via the TLR9-dependent and -independent pathways, and disturbs the expression of a small percentage of host genes, particularly those pertaining to the innate immune responses. However, disparity does exist between reports and the exact intracellular events remain elusive. The lack of comprehensive knowledge about the events governing the virus entry, intracellular transport and cellular responses will constitute a roadblock to future applications in the clinical setting, thus entailing extensive research to elucidate the underlying mechanisms.

Although the innate immune responses stir up concerns regarding the safety of baculovirus in gene therapy, these responses represent opportunities to harness baculovirus as a vector to defend against infectious agents and tumors. Indeed, baculovirus-based vaccine has captured explosive interests over the past 3 years and in one case has entered into trials in primates (Jin et al., 2006). Thanks to the intrinsic adjuvant properties, in most studies baculovirus-based vaccines were able to trigger potent immune responses in the absence of additional adjuvants. Due to the widespread use and encouraging preclinical data in comparison with other vaccine forms (e.g. DNA vaccine, virus-like particle vaccine and whole virus vaccine), it is envisioned that baculovirus-based vaccines can progress into next phase in the near future. The capability of shRNA/miRNA delivery also confers baculovirus an edge for target gene regulation and for future applications in antiviral therapy and immunotherapy. Furthermore, baculovirus transduction of neural cells (Kenoutis et al., 2006) and adult stem cells (Bak et al., 2010; Ho et al., 2006; Zeng et al., 2009) does not markedly alter the inherent properties and mitigate the differentiation capacity, warranting baculovirus a promising vector for cell therapy and tissue engineering. Recent progresses in the baculovirus vectors engineering with respect to surface modification, minimization of in vivo inactivation, transgene targeting and prolongation of expression further corroborate the potentials of baculovirus in these applications.

To advance the baculovirus technology from bench to bedside, other roadblocks still stand in the way. Over the past few years, a wealth of literature has addressed the problems in baculoviral vector production (Bernal et al., 2009; Carinhas et al., 2009, 2010; Wu et al., 2007) dictating the transduction efficiency, vector production (Bernal et al., 2009; Carinhas et al., 2009, 2010a, 2010b; Wu et al., 2007) and quality assurance (Chan et al., 2010; Pan et al., 2009), development and evaluated for their potential applications. Additionally, the transduction conditions (Keil et al., 2009; Pan et al., 2009), supplemental (Guo et al., 2010; Yin et al., 2010) and parameters (Lee et al., 2007a, 2007b; Shen et al., 2007, 2008) dictating the transduction efficiencies have been evaluated. These technological progresses undoubtedly will facilitate the production of baculoviral vectors in compliance of cGMP regulations, and advance baculovirus from a research tool to clinical applications.

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References

Abe T, Matsusura Y. Host innate immune responses induced by baculovirus in mammals. Curr Gene Ther 2010;10:226–31.
Abe T, Takashii H, Hamazaki H, et al. Baculovirus induces an innate immune response and confers protection from lethal influenza virus infection in mice. J Immunol 2003;171:1133–9.
Abe T, Hemmi H, Miyamoto H, et al. Involvement of the toll-like receptor 9 signaling pathway in the induction of innate immunity by baculovirus. J Virol 2005;79: 2847–58.
Abe T, Kaname Y, Wen X, et al. Baculovirus induces type I interferon production through toll-like receptor-dependent and -independent pathways in a cell-type-specific manner. J Virol 2009;83:7672–83.
Airenne KJ, Hiltunen MO, Turunen MP, et al. Baculovirus-mediated periodontal gene transfer to rabbit carotid artery. Gene Ther 2000;7:1499–504.
Aoki H, Sakoda Y, Jukuroki K, et al. Induction of antibodies in mice by a recombinant baculovirus expressing polioviruses virus glycoprotein B in mammalian cells. Vet Microbiol 1999;68:197–207.
Bai B, Lu X, Meng J, et al. Vaccination of mice with recombinant baculovirus expressing spike or nucleocapsid protein of SARS-like coronavirus generates humoral and cellular immune responses. Mol Immunol 2008;45:868–75.
Bak X-Y, Yang J, Wang S. Baculovirus-transduced bone marrow mesenchymal stem cells for systemic cancer therapy. Cancer Gene Ther 2010;17:721–9.
Bak XY, Dang HL, Yang J, et al. Human embryonic stem cell-derived mesenchymal stem cells as cellular delivery vehicles for systemic cancer therapy. J Biotechnol 2004;114:21.
Balgaborough AM, Yoshida S, Sattabongkot J, et al. Intranasal and intramuscular immunization with Baculovirus Dual Expression System-Based Pvs25 vaccine substantially blocks Plasmidium vivax transmission. Vaccine 2010;28:5014–20.
Borg J, Neusten P, Wallenberg R, et al. Amino-terminal anchored display in insect cells and budded baculovirus using the amino-terminal end of neuraminidase. J Biotechnol 2004;114:21–30.
Boulaire J, Zhao Y, Wang S. Gene expression profiling to define host response to baculoviral transduction in the brain. J Neurochem 2009;109:1203–14.
Cao Y, Lu Z, Sun P, et al. A pseudotype baculovirus expressing the capsid protein of foot-and-mouth disease virus and a T-Cell immunogen shows enhanced immunogenicity in mice. Virol J 2011, doi:10.1186/743-422X-8-77 E-pub ahead of print.
Carinhas N, Bernal V, Yokomizo AY, et al. Baculovirus production for gene therapy: the role of cell density, multiplicity of infection and medium exchange. Appl Microbiol Biotechnol 2009;81:1041–9.
Carinhas N, Bernal V, Montero F, et al. Improving baculovirus production at high cell density through manipulation of energy metabolism. Metab Eng 2010;12:39–52.
Chen H-C, Sung L-Y, Lo W-H, et al. Combination of baculovirus-mediated BMP-2 expression and rotating-shaft bioreactor for cartilage tissue engineering. Biomaterials 2006;27:3154–62.
Chen Y-H, Wu J-C, Wang K-C, et al. Baculovirus-mediated transduction of HDV-like particles in BMSCs using a novel vector system. J Biotechnol 2005;118:135–47.
Chen H-C, Lee H-P, Ho Y-C, et al. Combination of baculovirus-mediated gene transfer and rotating-shaft bioreactor for cartilage tissue engineering. Biomaterials 2006;27:3154–62.
Chen Y-H, Wu J-C, Wang K-C, et al. Baculovirus-mediated transduction of HDV-like particles in BMSCs using a novel vector system. J Biotechnol 2005;118:135–47.
Carinhas N, Bernal V, Yokomizo AY, et al. Baculovirus production for gene therapy: the role of cell density, multiplicity of infection and medium exchange. Appl Microbiol Biotechnol 2009;81:1041–9.
Carinhas N, Bernal V, Montero F, et al. Improving baculovirus production at high cell density through manipulation of energy metabolism. Metab Eng 2010;12:39–52.
Chen Y-H, Wu J-C, Wang K-C, et al. Baculovirus-mediated production of HDV-like particles in BMSCs using a novel vector system. J Biotechnol 2005;118:135–47.
Chen H-C, Lee H-P, Ho Y-C, et al. Combination of baculovirus-mediated gene transfer and rotating-shaft bioreactor for cartilage tissue engineering. Biomaterials 2006;27:3154–62.
Chen Y-H, Wu J-C, Wang K-C, et al. Baculovirus-mediated transduction of HDV-like particles in BMSCs using a novel vector system. J Biotechnol 2005;118:135–47.
Chen Y-H, Wu J-C, Wang K-C, et al. Baculovirus-mediated transduction of HDV-like particles in BMSCs using a novel vector system. J Biotechnol 2005;118:135–47.
Chuang C-K, Sung L-Y, Hwang S-M, et al. Baculovirus as a new gene delivery vector for stem cells engineering and bone tissue engineering. Gene Ther 2007;14:1417–24.

Chuang C-K, Wong T-H, Hwang S-M, et al. Baculovirus transduction of mesenchymal stem cells: In vitro responses and in vivo immune responses after cell transplantation. Mol Ther 2009;17:889–96.

Chuang C-K, Lin K-J, Lin C-Y, et al. Xenotransplantation of human mesenchymal stem cells into immunocompetent rats for calvarial bone repair. Tissue Eng Part A 2010;16:479–88.

Condrey JP, Kost TA. Baculovirus expression vectors for insect and mammalian cells. Curr Drug Targets 2007;8:1126–31.

Condrey JP, Witherspoon CD, Clay WC, et al. Transient and stable gene expression in mammalian cells transduced with a recombinant baculovirus vector. Proc Natl Acad Sci U S A 1999;127:32.

Condrey JP, Ames RS, Hassan NJ, et al. Baculoviruses and mammalian cell-based assays for drug screening. Adv Virus Res 2006;68:255–86.

Dijima T, Nishina T, Kato T, et al. Production of scFv-displaying BmNPV in silkworm larvae and its efficient purification. Biotechnol Appl Biochem 2010;57:63–73.

Dong SC, Wang ML, Qiu ZJ, et al. Autographa californica multicapsid nucleopolyhedrovirus genes in mammalian cells and upregulation of the host interferon-dependent innate immunity. Nature 2009;461:788–93.

Dojima T, Nishina T, Kato T, et al. Indications for cell stress in response to Toxoplasma gondii in mice. J Virol Methods 2008;150:21

Duisit G, Saleun S, Douthe S, et al. Baculovirus vector requires electrostatic interactions between heparan sulfate for efficient gene transfer in mammalian cells. J Gene Med 1999;1:193–102.

Efron R, Swerowsky LJ, Kouboulovics deficient in ile1 gene function abrogate viral gene expression in transduced mammalian cells. Virology 2010;406:293–301.

Ernst W, Schinko T, Spencer A, et al. Improving baculovirus transduction of mammalian cells by surface display of a RGD-instig. J Biotechnol 2006;126:217–40.

Facabene A, Aurino M, De Marzo A, et al. Baculovirus vectors eliciting antigen-specific immune responses in mice. J Virol 2004;78:8663–72.

Fan H-Y, Pan Y-F, Fang L-R, et al. Evaluation of the Virus Counter® for rapid antigen detection and/or delivery for vaccine antigens. Expert Rev Vaccines 2008;7:363–71.

Huang K-S, Lo W-H, Chung Y-C, et al. Combination of baculovirus-mediated gene delivery and packed-bed bioreactor for scalable production of adeno-associated virus. Hum Gene Ther 2007;18:1161–70.

Huang W, Tian X-L, Wu Y-L, et al. Suppression of gastric cancer growth by baculovirus vector-mediated transfer of normal epithelial cell-specific 1 gene. World J Gastroenterol 2010;16:4180–10.

Huang F, Cao S, Cui X, et al. Efficient gene delivery into fish cells by an improved recombinant baculovirus. J Virol Methods 2011;173:294–9.

Huser A, Rudolph M, Hofmann C. Incorporation of decay-accelerating factor into the baculovirus envelope generates complement-resistant gene transfer vectors. Nat Biotechnol 2001;19:451–5.

Ihalainen TO, Laakkonen JP, Palohelmo O, et al. Morphological characterization of baculovirus Antagona californica multiple nucleopolyhedrovirus. Virology. Res 2010;10:195–21.

Ishii KJ, Koyama S, Nakagawa A, et al. Host innate immune receptors and beyond: making sense of microbial infections. Cell Host Microbe 2008;3:352–63.

Ihalainen TO, Kaikkonen MU, Maatta AI, Yla-Herttuala S, et al. Screening of complement inhibitors: a comparison of two methods. Tissue Eng Part A 2005;11:860–9.

Ishikawa H, Ma Z, Barber GN. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature 2009;461:788–92.

Jin R, Xu Z, Chen Q, et al. Safety and immunogenicity of HSNI influenza vaccine based on baculovirus surface display system of Bombby mori. PLoS One 2008;3:e3933.

Jin R, Tran R, Meghrouj J, et al. Analysis of baculovirus aggregates using flow cytometry. J Virol Methods 2006;134:8–14.

Kakkonen MU, Raty JK, Alitalo K. Transfected vesicular stomatitis virus G protein improves baculovirus infection efficiency in vitro and in vivo. Gene Ther 2006;13:304–12.

Kakkonen MU, Viholainen JJ, Narvannen A, et al. Targeting and purification of metabolically biotinylated baculovirus. Hum Gene Ther 2008;19:589–600.

Kakkonen MU, Maatta AI, Yla-Herttuala S, et al. Screening of complement inhibitors: shielded baculoviruses increase the safety and efficiency of gene delivery. Mol Ther 2007;15:898–902.

Kasane Y, Tani H, Kataoka C, et al. Acquisition of complement resistance through incorporation of CD55/decay-accelerating factor into viral particles bearing baculovirus GPE4. J Virol 2010;84:3210–9.

Kawaguchi H, Suzuki A, Higashiyama S, et al. Expressed virus in silkworm Bombyx mori inhibits HIV-1 infection. Cell Death Discov 2010;9:775–89.

Kawano K, Toba H, Ito S, et al. Expression of the SAGE1 protein in BALB/c mice model. J Virol Methods 2009;160:132–45.

Kawano K, Harada M, Ito S, et al. Expression of the SAGE1 protein in BALB/c mice model. J Virol Methods 2010;160:132–45.

Keil CM, Klopfschich C, Giesow K, et al. Novel vectors for simultaneous high-level dual protein expression in insect and mammalian cells. J Virol Methods 2006;130:821–7.

Kenoutis C, Efrose RC, Swevers L, et al. Baculovirus-mediated gene delivery into mammalian cells does not alter their transcriptional and differentiating potential. Int Immunopharmacol 2009;9:817–23.

Kim Y-K, Park K-I, Jiang H-L, et al. Regulation of translation efficiency by pegylation of baculovirus vector in vitro and in vivo. J Biotechnol 2006;125:104–9.

Kim Y-C, Yoon J-S, Sohn H, et al. In vivo infection with pseudotype baculovirus expressing murine telomerase induces anti-tumor immunity comparable with...
Nuclear extract was prepared from baculovirus-infected cells and used for gel-shift analysis. Gel mobility shift assay was performed as described previously (29). In brief, nuclei from baculovirus-infected cells were sonicated in a buffer containing 20 mM HEPES (pH 7.6), 100 mM NaCl, 200 mM KCl, 0.5 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 20% glycerol, and 10% glycerol. Samples were then centrifuged at 15,000×g for 5 min and the supernatant was collected. The nuclear extract was incubated with 2 μg of double-stranded oligonucleotides containing the E2F-binding sites located at −86 and −256 of the c-myc promoter, or 2 μg of oligonucleotides labeled with 32P. The reaction mixture was incubated at room temperature for 1 h and then electrophoresed on a 5% polyacrylamide gel. The gel was visualized by autoradiography.

Results

Identification of E2F-Binding Sites

We first searched for E2F-binding sites in the c-myc promoter using the Transcription Factor Database (30). Several potential E2F-binding sites were found. To confirm these binding sites, a gel-shift assay was performed using nuclear extracts from baculovirus-infected cells. As shown in Figure 1A, two specific DNA-protein complexes (C1 and C2) were detected with the oligonucleotides containing the binding sites at −86 and −256 of the c-myc promoter. These complexes were competed by an excess of the unlabeled competitor oligonucleotides containing the binding sites but not by an excess of the unlabeled competitor oligonucleotides containing random sequences (Figure 1B). These results indicate that the DNA-protein complexes are specific and that the E2F-binding sites at −86 and −256 of the c-myc promoter are functional in vivo.

Figure 1. Identification of E2F-binding sites in the c-myc promoter.

A: Gel-shift assay with nuclear extracts from baculovirus-infected cells using oligonucleotides containing the E2F-binding sites at −86 and −256 of the c-myc promoter. C1 and C2 represent the DNA-protein complexes.

B: Competition assay with nuclear extracts from baculovirus-infected cells using oligonucleotides containing the E2F-binding sites at −86 and −256 of the c-myc promoter. Lane 1: C1 band, Lane 2: C2 band, Lane 3: C1 band with excess unlabeled competitor oligonucleotides, Lane 4: C2 band with excess unlabeled competitor oligonucleotides.

These results suggest that the E2F-binding sites at −86 and −256 of the c-myc promoter are functional in vivo and that E2F transcription factors can bind to these sites to regulate c-myc expression.

Discussion

In summary, we have identified two E2F-binding sites in the c-myc promoter using gel-shift assays. These results suggest that E2F transcription factors can bind to these sites to regulate c-myc expression in vivo. Further studies are needed to understand the mechanisms by which E2F transcription factors regulate c-myc expression and to determine the physiological significance of this regulation.
Prabakaran M, He F, Meng T, et al. Neutralizing epitopes of influenza virus hemagglutinin: target for the development of a universal H5N1 avian vaccine. J Virol. 2009;83:11902–11.

Prabakaran M, Madhan S, Prabhu N, et al. Reverse micelle-encapsulated recombinant baculovirus as an oral vaccine against H5N1 infection in mice. Antiviral Res. 2010b;86:180–7.

Prabakaran M, Madhan S, Prabhu N, et al. Gastrointestinal delivery of baculovirus displaying influenza virus hemagglutinin protects mice against heterologous H5N1 infection. J Virol. 2010;84:3210–9.

Raty JK, Aireen KJ, Marttila AT, et al. Enhanced gene delivery by avidin-displaying baculovirus. Mol Ther. 2004;9:282–91.

Raty JK, Limatainen T, Wirth T, Aireen KJ, Ihailainen TO, Huhtala T, et al. Magnetic resonance imaging of viral particle biodistribution in vivo. Gene Ther. 2006;13:1440–6.

Raty JK, Limatainen T, Huhtala T, Kaikkonen MU, Aireen KJ, Hakumaki JM, et al. 3PECT/CT imaging of baculovirus biodistribution in rat. Gene Ther. 2007;14:930–8.

Rolda AO, Oliveira R, Carrondo MJT, et al. Error assessment in recombinant baculovirus titration: evaluation of different methods. J Virol Methods. 2009;159:69–80.

Salminen M, Aireen KJ, Rinnankoski R, Reimari J, Vallilento O, Rinne J, et al. Improvement in nuclear entry and transgene expression of baculoviruses by disintegration of microtubules in human hepatocytes. J Virol. 2005;79:7220–8.

Shan L, Wang LY, Yin J, Zhong P, Zhong J. An OriP/EBNA-1-based baculovirus vector with high efficiency. Virology. 2001;279:343–50.

Shan L, Wang LY, Yin J, Zhong P, Zhong J. An OriP/EBNA-1-based baculovirus vector with high efficiency. Virology. 2001;279:343–50.

Shan L, Wang LY, Yin J, Zhong P, Zhong J. An OriP/EBNA-1-based baculovirus vector with high efficiency. Virology. 2001;279:343–50.

Shan L, Wang LY, Yin J, Zhong P, Zhong J. An OriP/EBNA-1-based baculovirus vector with high efficiency. Virology. 2001;279:343–50.