Establishment and characterization of three embryonic cell lines of beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae)

Rui Su · Gui-Ling Zheng · Fang-Hao Wan ·
Chang-You Li

Received: 16 November 2014 / Accepted: 4 May 2015 / Published online: 22 May 2015
© Springer Science+Business Media Dordrecht 2015

Abstract Three cell lines (QAU-Se-E-1, -2 and -3, or Se-1, -2 and -3 for short) were established from eggs of beet armyworm (*Spodoptera exigua*) that have been passaged stably for more than 60 times in TNM-FH medium supplemented with 10 % fetal bovine serum. The cell lines consisted of round and spindle-shaped cells. The round cells accounted for 96.82, 84.34 and 83.16 % of the cells in the three cell lines, respectively, with cell diameters of 16.21 ± 0.72 , 15.63 ± 0.58 and 13.06 ± 0.44 μm . Random amplified polymorphic DNA and analysis of the CO I gene showed that the three cell lines were all derived from *S. exigua*. Growth curves at passage 30 were determined and the results showed that the cell population doubling times were 59.03, 49.08 and 49.91 h, respectively. The three cell lines can be infected by *S. exigua* multiple nucleopolyhedrovirus (SeMNPV). Se-3 was extremely susceptible to the virus with an infection rate of 97.52 % 4 days after the inoculation and produced 2.02×10^6 OBs per mL of culture. Flow cytometry

analysis showed that some of Se-1 and Se-2 cells had apoptosis after infection, whereas Se-3 cells did not. Bioassays showed that the virulence of the SeMNPV proliferated from Se-3 was similar to that from the insect with LC_{50} of 5.55×10^5 and 2.64×10^5 OBs/mL. Therefore, the cell lines can be used to study the SeMNPV-host interactions and mechanisms underlying the interactions.

Keywords *Spodoptera exigua* · Embryonic cell line · Mitochondrial CO I gene · Nucleopolyhedrovirus · Apoptosis

Introduction

With the development of insect cell culture technology, cultured insect cells have been widely used in many scientific research areas, and playing a vital role in basic researches such as genetics and molecular biology, or applied sciences such as agriculture and medicine (Smaghe et al. 2009). In comparison with whole insects, cultured cells have a number of advantages such as fast and uniform growth, in addition, their environmental conditions can be more readily controlled. Therefore, they are especially useful in the study of the proliferation, virulence determination, and mechanism of action of biological pesticides. Beet armyworm (*Spodoptera exigua*) is a global and important agricultural pest, causing serious damage in Asia, Europe, America, Oceania, and Africa

R. Su · G.-L. Zheng · F.-H. Wan · C.-Y. Li (✉)
Key Lab of Integrated Crop Pest Management of
Shandong Province, College of Agronomy and Plant
Protection, Qingdao Agricultural University,
Qingdao 266109, Shandong, People's Republic of China
e-mail: 1066406555@qq.com; cyli@qau.edu.cn

F.-H. Wan
State Key Laboratory for Biology of Plant Diseases and
Insect Pests, Institute of Plant Protection, Chinese
Academy of Agricultural Sciences (CAAS),
Beijing 100193, People's Republic of China

(Bianchi et al. 2002; Sumerford 2003; Saeed et al. 2010; Underwood 2011). Since its outbreak in China in 1986, the insect is epidemic and expanding in more than 20 provinces, posing serious threat to agricultural production (Si et al. 2012). Therefore, it is extremely important to find an effective and safe solution to the pest. *S. exigua* multiple nucleopolyhedrovirus (SeMNPV) is a biological insecticide to control beet armyworm. SeMNPV is highly host-specific, effective, non-polluting and safe to the natural enemies, human and other higher animals. It is not easy to produce resistance to the virus which is ideal for pest control. In vitro cultured insect cells, are the most ideal tools for virology and platform for development of viral and other insecticide (Goodman et al. 2001b; Decombel et al. 2005). Establishment of cell lines susceptible to the virus will be of great significance for biological control of beet armyworm. In this study, three cell lines derived from the embryonic tissues of beet armyworm were established and characterized. These cell lines will be very useful for the development of SeMNPV-based insecticides and physiological study of the insect.

Materials and methods

Primary cell culture

Spodoptera exigua eggs laid within 12 h were obtained from the colony reared on an artificial diet in the laboratory. About 500 eggs were surface-sterilized in 10 % sodium hypochlorite and subsequently in 75 % ethanol for 5 min each under sterile conditions, then rinsed three times with sterile distilled water, and once with TNM-FH medium (HyClone, South Logan, UT, USA) containing 15 % heat-inactivated (56 °C for 30 min) fetal bovine serum (FBS) (TransGen Biotech, Beijing, China). The eggs were transferred into a 100 µm cell strainer and immersed into 6 mL of TNM-FH medium containing 15 % FBS and 1 % gentamicin (Nacalai Tesque, Nakagyo-ku, Kyoto, Japan) in a Petri dish, ground using a sterile rubber-policeman to rupture the eggs. The cells were allowed to pass through the mesh to disperse into culture medium. 1.5 mL cell filtrates were transferred to a 25 cm² flask containing 3.5 mL of TNM-FH medium with 15 % FBS and 1 % gentamicin to which about

30 mg/mL phenylthiourea (Sigma, St. Louis, MO, USA) was added to inhibit tyrosinase activity (Eide et al. 1975), and cultured at 27 °C incubator. The cultures were regularly observed under an inverted microscope. In the early stage of primary culture, medium was replaced every 2 weeks with 2.5:2.5 (v/v) ratio (removed 2.5 mL of cell suspension and added 2.5 mL of fresh medium), and in the later stage, the medium was refreshed every week in different ratio according to cell growth.

Cell passage

When the primary attached cultured cells were grown to confluency, they were suspended by gently pipetting and subcultured to new flask with 2.5:2.5 (v/v) ratio (transferred 2.5 mL of cell suspension to a flask containing 2.5 mL of fresh medium). Thereafter, the cells were subcultured in 2:3 (v/v) ratio. At about 10 passages, the cells were gradually stabilized, the serum concentration in the culture medium was reduced to 10 %, and the cells were subcultured in 1:4 (v/v) ratio. After 20 passages, the cells cultured at 27 °C could be passaged once every 4–5 days in TNM-FH medium containing 10 % FBS, indicating that the cell lines were successfully established. They were given names accordingly, observed and photographed for morphology under an IX71 inverted microscope. For each cell line, three randomly selected fields were selected and size of 30 cells in each field were measured.

RAPD fingerprinting

Genomic DNA from the cell lines and insect eggs were extracted using the genomic DNA purification kit (TOYOBO LIFE SCIENSE, Osaka, Japan) according to the manufacturer's instructions. RAPD analysis (Meng et al. 2008) was carried out using the following random primers: primers OPU-05 (CCC AGT CAC T), OPU-09 (CCA CAT CGG T) (Wu and Wang 2006), No. 2 primer (CCG CAT CTA C) (Kawai and Mitsuhashi 1997) and *T. ni* specific primer (TTG CTG TCC) (Li et al. 2003) synthesized a Commercial Company (Sangon Biotech, Shanghai, China). *T. ni* cell line BTI-Tn5B1-4 (High Five) and *S. frugiperda* cell line Sf-9 provided by Dr. Granados at BTI of Cornell University were used as the control.

Amplification and comparison of mitochondrial CO I gene

CO I gene in genomic DNA from the cell lines and insect eggs was amplified using universal primers HCO 2198 and LCO 1490 (Folmer et al. 1994) in a reaction containing 5 μ L of 10 $\times$ PCR Buffer, 4 μ L of 2.5 mmol/L dNTPs, 1 μ L each of 10 μ mol/L primers, 2.5 U of Taq DNA polymerase (TransGen Biotech, Beijing, China), 50 ng genomic DNA templates, and sterile distilled water filled up to 50 μ L. The reaction conditions were pre-denaturing at 94 °C for 5 min, followed by 30 cycles of denaturing at 94 °C for 1 min, annealing at 54 °C for 1 min and extending at 72 °C for 2 min, with final extending at 72 °C for 5 min. The PCR products were examined by agarose gel electrophoresis, and sequenced (Sangon Biotech, Shanghai, China).

Determination of cell growth curve and population doubling time

Cells in the logarithmic growth phase at the 30th passage were counted with a hemocytometer, diluted to 2×10^5 cells/mL with the culture medium, inoculated onto wells of 24 well plate (1 mL/well) and cultured at 27 °C. Cells from three wells were taken and counted every day to calculate the cell density and plot the growth curve. The growth curve equation at the logarithmic phase was fit and the population doubling time was calculated using the SPSS 20.0 software (Norusis 2012).

Determination of susceptibility to virus

1 mL of cells in the logarithmic growth phase was inoculated onto 24 well plates at a density of 2×10^5 cells/mL and cultured at 27 °C to allow the cells to attach to the bottom of wells. SeMNPV provided by Dr. Ping Wang at Cornell University, was added to the cells at a multiplicity of infection (MOI) of ten after removing the culture medium. The experiments were repeated three times. After the inoculation, the plates were slowly shaken on a vertical shaker for 2 h for virus adsorption. Then the virus suspension was removed, 1 mL of fresh medium was added and the plates were cultured at 27 °C in an incubator. The infection was examined under an inverted microscope and

photographed. Numbers of total cells and infected cells in three randomly selected views in each well were counted and used to calculate the infection rate. The cells were then harvested from the plates and lysed with an ultrasonicator to release virus polyhedra and counted with a hemocytometer to determine the production of occlusion bodies (OBs).

Detection of apoptosis induced by SeMNPV using AnnexinV/PI double staining

Cells in logarithmic growth phase were seeded in 25 cm² flask at the density of 2×10^5 cells/mL. The cells were inoculated with SeMNPV virus as described in “[Determination of susceptibility to virus](#)” section. After incubation for 48 h, about 10^6 cells were collected by centrifugation and stained with Annexin V-FITC and PI according to the protocol provided in the TransDetectTM Annexin V-FITC/PI Cell Apoptosis Detection Kit (TransGen Biotech, Beijing, China). The samples were analyzed by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA), and data were processed by FlowJo 7.6.1 software.

Comparison of virulence for virus proliferated in cells and insects

SeMNPV from susceptible cell cultures and insects diluted to 2×10^8 , 2×10^7 , 2×10^6 , 2×10^5 , 2×10^4 OBs/mL and sterile distilled water as control were used to feed 30 4th instar larvae. The experiments were repeated three times and carried out using 24 well plates with about 30 mm³ diet in each well, uniformly added with 10 μ L of virus suspension. The insects tested were reared in an incubator at 27 ± 1 °C in a light: dark cycle of 12 h: 12 h. Once the diet containing virus suspension was consumed, the insects were transferred to different rearing boxes at suitable density to continue feeding with fresh feed. The number of diseased insects were recorded every day till the death or pupation. The corrected mortality was calculated using conventional biological statistics method. The linear regression equation between the mortality probability and logarithmic virus concentration was obtained (Liu et al. 2010), as well as LC₅₀ values and their 95 % confidence limits using the Probit method (Norusis 2012).

Results

Primary culture and establishment of cell lines

Primary culture of embryonic cells was initiated in May, 2013 and the cells were in good state at the early stage and formed netted structures 2 weeks after culture (Fig. 1a). Later on, the cell numbers were not increased significantly but the net structures grew quickly and filled up the bottom of the flask. About 2 months after primary culture, periodical twitches at some nodes were observed, showing that the cells were very active. New cells were seen growing out of the matrix to cover the reticular tissue (Fig. 1b). With increase in proliferated cells, the bottom of the flask was fully covered with cells and a few of them

suspended in culture medium (Fig. 1c). After more than 3 months of primary culture, the embryonic cells were subcultured. The cells in the first passage grew slowly with diverse morphology and some reticular structure. About 2 weeks later, the cells were subcultured again. After that, the cells were subcultured at intervals and ratio determined based on their growth. The cells became stabilized after 10 passages (Fig. 1d, e, f). Then the FBS content in culture medium was dropped to 10 % and the cells were subcultured every 4 or 5 days at a ratio of 1:4 (v/v). Three cell lines were obtained and were named QAU-Se-E-1, QAU-Se-E-2 and QAU-Se-E-3 (or Se-1, Se-2 and Se-3 for short).

The three cell lines were adherent cells and had been successfully passaged for more than 60 times so far. However, the morphology of cells in these cell

Fig. 1 Phase contrast micrographs of cell lines and its primary culture. Showing *Spodoptera exigua* cells growing out of the matrix and increased to cover the bottom of the flask. At passage 10, two major cell types, round cells and spindle-shaped cells were found in three cell lines. **a–c** Primary culture of *S. exigua* embryonic cells at different times. **a** Two weeks, **b** 50 days, **c** 100 days. **d–f** Three cell lines at passage 10. **d** Se-1, **e** Se-2, **f** Se-3. Bar 50 μ m

lines were different. Cells of Se-1 were larger and mostly round cells (96.82 %) with diameter of $16.21 \pm 0.72 \mu\text{m}$ and few spindle-shaped cells (about 3.18 %) with size of $(44.31 \pm 4.78) \times (12.79 \pm 1.27) \mu\text{m}$. In Se-2, about 84.34 % were round cells with diameter of $15.63 \pm 0.58 \mu\text{m}$, and about 15.66 % were spindle-shaped cells with size of $(26.99 \pm 1.45) \times (10.37 \pm 0.40) \mu\text{m}$. The Se-3 consisted mainly of round cells (about 83.16 %) with diameter of $13.06 \pm 0.44 \mu\text{m}$ and some spindle-shaped cells (about 16.84 %) with size of $(39.68 \pm 3.66) \times (10.69 \pm 0.71) \mu\text{m}$.

RAPD fingerprint analysis

The sizes of amplified DNA polymorphic fragments with the four primers were between 500 and 4000 bps (Fig. 2). Using primer No. 2, four specific bands (2500, 1700, 940, and 520 bps) were amplified from the three cell lines and beet armyworm eggs and four specific bands (2640, 1900, 1550, and 830 bps) were amplified using primer *T. ni*, six specific bands (2520, 1940, 1640, 940, 770, and 650 bps) were obtained using primer OPU-05 and another six specific bands (2300, 2000, 1780, 1000, 820, and 580 bps) were generated using primer OPU-09. These bands were obviously different from those amplified from Sf-9 and BTI-Tn5B1-4 cells, and almost the same between the cell lines and beet armyworm eggs, indicating that

the three cell lines and beet armyworm have the same genetic background.

Identification of the cell lines by CO I gene

Fragments of the CO I gene were cloned and sequenced from the three cell lines and the beet armyworm eggs. The length of the fragments was 629 bps with identical nucleotide sequence (the sequences from beet armyworm eggs and cell line Se-1 have been submitted to NCBI with the accession numbers of KM491550 and KM491551). Alignment of the sequence with the sequence of beet armyworm CO I gene (JX316220) in deposited in NCBI showed that they are 100 % identical, indicating that the cell lines were derived from beet armyworm.

Cell growth curve and population doubling time

The growth curves of Se-1, Se-2 and Se-3 at the 30th passage are shown in Fig. 3. When compared, the three growth curves were similar and reached logarithmic growth phase 3 days after passage, when the number of cells significantly increased. By the 7th day, the maximum cell density was reached, the density of Se-2 cells was about 2.32×10^6 cells/mL, and the maximum cell densities of Se-1 and Se-3 were 1.80×10^6 and 2.04×10^6 cells/mL, respectively. After 7 days of culture, with the increase of cell

Fig. 2 RAPD profile of *S. exigua* eggs and several insect cell lines. RAPD profile of *S. exigua* eggs and several insect cell lines using different primers. **a** Primer No. 2; **b** *T. ni* specific Primer; **c** Primer OPU-05; **d** Primer OPU-09. The sample order: M. Trans 2 K PlusIIDNA Marker, used as size markers and are indicated on the sides in bp; 1 *S. exigua* eggs; 2 Se-1 cells; 3 Se-2 cells; 4 Se-3 cells; 5 BTI-Tn5B1-4 cells; 6 Sf-9 cells

density, the nutrition was insufficient and metabolites were accumulated, resulting in cell lysis and death. As a result, there were a large number of particles and cell debris in the culture medium, and the cell number decreases gradually. Growth curve data were fitted into exponential models for the three cell lines using the SPSS 20.0 software. The calculations showed that the cell lines had population doubling times of 59.03, 49.08, and 49.91 h, respectively.

Determination of susceptibility to SeMNPV

There were slight differences in the three cell lines in response to SeMNPV infection (Fig. 4). In the initial stage, the three cell lines showed slowed growth and obvious sign of NPV infection 3 days after the infection. For Se-1, most cells had typical

Fig. 3 The growth curves of *S. exigua* cell lines Se-1, Se-2, and Se-3. The cells (at 30th passage) were cultured at 27 °C in TNM-FH medium supplemented with 10 % FBS. Each point represents the cell density at the indicated time after seeding. The error bars indicate the standard deviation of three independent trials

morphological changes of infection, such as nuclear enlargement and occurrence of a large number of polyhedra in the nucleus. Some cells had apoptosis, producing a large number of apoptotic bodies. For Se-2, diseased cells tended to aggregate and their enlarged nuclei were filled up with viral inclusion bodies as seen under the microscope. Some of the cells were also apoptotic. Se-3 had higher susceptibility to the virus with decentralized cells. No apoptosis was seen due to the infection. In the later stage of infection, more and more cells died and the contour of the cells became blurred with rough edge. The dead cells were ruptured and suspended and the medium was full of cell debris and polyhedral.

The infection rates three and four days after virus inoculation are listed in Table 1. The yields of occlusion body recovered from the culture on the 4th day were determined.

As shown in Table 1, Se-1 was not susceptible to SeMNPV with an infection rate of 10.39 % 4 days after infection and occlusion body yield of 9.25×10^5 OBs/mL; Se-2 was highly susceptible to SeMNPV with a 4-day infection rate of 93.55 % and occlusion body yield of 1.87×10^6 OBs/mL; Se-3 was even more susceptible with a 4-day infection rate of 97.52 % and the highest occlusion body yield of 2.02×10^6 OBs/mL.

Apoptosis induced by SeMNPV in three cell lines

By double staining with AnnexinV-FITC/PI and flow cytometry analysis, in Se-1 cells, the early apoptotic cells and necrotic cells were 40.3 and 33.9 %, respectively.

Fig. 4 Bright field micrographs of cell lines Se-1, Se-2, and Se-3 infected with SeMNPV at 4 days post infection. Showing slight differences in the three cell lines in response to SeMNPV

infection. Three cell lines were infected with SeMNPV, respectively. **a** Se-1, **b** Se-2, **c** Se-3. OBs viral occlusion bodies, ABs apoptotic bodies. Bar 50 μm

Table 1 Infection rate of the cell lines to SeMNPV and occlusion body yield

Virus	Cell line	Infection rate (%) at day 3	Infection rate (%) at day 4	Occlusion body yield (Os/mL)
SeMNPV	Se-E-1	10.39 ± 3.42	27.83 ± 4.44	9.25 × 10 ⁵
	Se-E-2	56.26 ± 4.75	93.55 ± 2.49	1.87 × 10 ⁶
	Se-E-3	88.39 ± 3.34	97.52 ± 1.39	2.02 × 10 ⁶

respectively, after infection with SeMNPV for 48 h (Fig. 5a). In Se-2 cells, the percentages were 32.1 and 26.2 %, respectively (Fig. 5b). These results suggest that SeMNPV had certain ability to induce apoptosis in Se-1 and Se-2 cells. However, only a very small number (8.60 % at 48 h) of Se-3 cells were apoptotic after SeMNPV infection. Some of the cells died directly due to virus infection with the mortality of 49.4 % (Fig. 5c). Combined with the virus sensitivity tests, the result showed that large numbers of Se-1 and Se-2 cells were apoptotic after SeMNPV infection in addition to typical polyhedrosis, while Se-3 cells had higher infection rate but fewer apoptosis.

Comparison of virulence of SeMNPV from the cell lines and insects

Bioassays showed that the insects began to become sick 3–4 days after inoculation with SeMNPV from the Se-3 cells at 2×10^8 OBs/mL. The incidence rate increased during the following 2–3 days. By the 10–12th day, all inoculated insects were either dead or pupated. The final corrected mortalities for insects inoculated with SeMNPV from Se-3 or the insects

were between 16.67 and 98.81 %, and 23.17 and 98.81 %, respectively. The linear regression equations between the mortality probability and logarithmic virus concentration are $y = 0.650x + 0.757$ ($R^2 = 0.982$) and $y = 1.264x + 0.689$, ($R^2 = 0.986$), respectively.

As shown in Table 2, the SeMNPV from the cell line Se-3 was similar to the virulence of the SeMNPV from insects.

Discussion

Beet armyworm is a well known and globally distributed pest, which is polyphagous, overeating and intermittent occurrence. In recent years, a number of beet armyworm cell lines have been established from embryos (Goodman et al. 2001a), hemocytes (Yasunaga-Aoki et al. 2004), neonate larval tissues (Weng et al. 2009), fat bodies (Zhang et al. 2006, 2009) and pupal tissues (Zhang et al. 2012; Wu et al. 2012). In this study, three cell lines Se-1, Se-2 and Se-3 were established from beet armyworm embryos. RAPD fingerprint analysis showed that the three cell

Fig. 5 The detection of apoptosis by annexin V-FITC/PI. Showing flow cytometry quadrants of apoptosis in Se-1, Se-2 and Se-3 cells induced by SeMNPV for 48 h. Q1 (Annexin-V-/PI+), mechanical damaged cells; Q2 (Annexin-V+/PI+), late

apoptotic and necrotic cells; Q3 (Annexin-V+/PI-), early apoptotic cells; Q4 (Annexin-V-/PI-), viable cells. **a–c** quadrantal diagrams of cells infected with SeMNPV. **a** Se-1, **b** Se-2, **c** Se-3

Table 2 Virulence of SeMNPV from the cell lines and insects

Virus	Source	LC ₅₀ (OBs/mL)	95% confidence limit (OBs/mL)	
			Upper	Lower
SeMNPV	Insect	2.64×10^5	4.49×10^5	1.43×10^5
	Se-3 cells	5.55×10^5	8.89×10^5	3.29×10^5

lines were derived from beet armyworm. In addition, the sequencing of mitochondrial CO I gene from the cell lines and the eggs confirmed that their sequences are the same. The CO I gene has been selected as DNA barcodes (Hebert et al. 2003) and has been widely used in molecular identification of insects, particularly Lepidoptera insects. In recent years, this technology has also been applied to the identification of origin of cell lines. For example, the CO I gene has been used to confirm the source tissues from which cell lines were established such as *Apis mellifera* (Hymenoptera: Apidae) embryonic tissue (Goblirsch et al. 2013), newly hatched larvae of *Papilio demoleus* Linnaeus (Lepidoptera: Papilionidae) (Ding et al. 2013) and embryonic tissue of *Gampsocleis gratiosa* (Orthoptera: Tettigoniidae) (Zhang et al. 2011). This in turn confirms its practicability and reliability. Using this method, as the supplement of RAPD analysis, we confirmed that the cell lines obtained in the study were indeed derived from beet armyworm.

Se-2 and Se-3 obtained in the study were highly susceptible to SeMNPV, and the virulence of SeMNPV from Se-3 was similar to that from the insects. Therefore, the cell line can be used to proliferate SeMNPV for production of viral insecticide. The studies have shown that cell lines from different tissues have different characteristics, and are highly variable in the susceptibility to virus (Hink et al. 1991). Beet armyworm cell lines from different tissues have been shown to differ in their susceptibility to virus. For example, the susceptibility of the cell lines from newly hatch larvae to SeMNPV was 73 ± 8 % (Hara et al. 1993, 1995), from fat body and ovarian tissues were 50 and 80 %, respectively (Zhang et al. 2006, 2012). And cell lines established from beet armyworm pupae had higher susceptibility to virus as 96 ± 1 % (Wu et al. 2012). In our study, the highest susceptibility to virus is 97.52 % in Se-3 cell line obtained from beet armyworm embryo. Wu et al. (2012) found that cell lines derived from pupa could have higher susceptibility to virus. Our work showed

that the two beet armyworm embryonic cell lines are highly susceptible to the virus, as well as other insect embryonic cell lines. For instance, the silkworm embryonic cell line (Li et al. 2011) is 91.3 % susceptible to BmNPV. *Helicoverpa armigera* embryonic cell line is more than 90 % susceptible to HaSNPV (Sudeep et al. 2002). Goodman et al. (2001b) found the *S. exigua* embryonic lines SeE1, SeE4, and SeE5 produced $8\text{--}14 \times 10^6$ TCID₅₀/mL in ExCell 401 containing 10 % FBS. These results demonstrate that embryonic tissue has high potential to establish cell lines that are susceptible to virus.

Although the three cell lines established in this study have exactly the same genetic background, they showed some differences in biological characteristics, which might be due to different differentiation of the immature stem cells because of small variations in the compositions of the culture medium such as growth factors during the embryonic cell culture process (Hakim et al. 2009), leading to different physiological characteristics. In the response to SeMNPV infection, apoptosis was observed in Se-1 and Se-2, but not in Se-3. Earlier study has reported SeMNPV-induced apoptosis in some cells (Ishikawa et al. 2003), which suggests that whether apoptosis occurs or not after virus infection, is mainly decided by the physiological characteristics of the cell itself. This may be resulted from the interactions between the virus and cells, and may be associated with some regulatory mechanisms generated under the stimulation of virus infection in the cells. In addition, induction of apoptosis by baculovirus is often happened in baculovirus infection of heterologous insect cells. For example, AcMNPV is shown to induce apoptosis in embryonic cells of America armyworm, *Pseudaletia unipuncta* (Wang et al. 1999), cells of spruce budworm, *Choristoneura fumiferana* (Palli et al. 1996) and epidermal cells HaEPi of cotton bollworm, *Helicoverpa armigera* (Shao et al. 2008). Little has been reported about apoptosis induced by homologous virus. In this study, we found apoptosis in the cell lines induced by

homologous virus. This findings lay the foundation for further study of the different mechanisms between homologous or heterologous virus and host cells. At the same time, the three cell lines are also ideal for further study on insecticidal mechanisms of SeMNPV and the mechanisms of cell apoptosis.

Acknowledgments This work was supported by the National Natural Science Foundation of China (31272376), Graduate Innovation Project of Qingdao Agricultural University (QYC201307), and Tai-Shan Scholar Construction Foundation of Shandong Province, China.

References

- Bianchi FJ, Vlcek JM, Werf WD (2002) Evaluation of the control of beet armyworm, *Spodoptera exigua*, with baculoviruses in greenhouses using a process-based simulation model. *Biol Control* 24:277–284
- Decombel L, Tirry L, Smagghe G (2005) Action of 24-epibrassinolide on a cell line of the beet armyworm, *Spodoptera exigua*. *Arch Insect Biochem Physiol* 58:145–156
- Ding WF, Feng Y, Zhang X, Li X, Wang CY (2013) Establishment and characterization of a cell line developed from the neonate larvae of *Papilio demoleus* Linnaeus (Lepidoptera: Papilionidae). *In Vitro Cell Dev Biol Anim* 49:108–113
- Eide PE, Caldwell JM, Marks EP (1975) Establishment of two cell lines from embryonic tissue of the tobacco hornworm, *Manduca sexta* (L.). *In Vitro* 11:395–399
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol* 3:294–299
- Goblirsch MJ, Spivak MS, Kurti TJ (2013) A cell line resource derived from honey bee (*Apis mellifera*) embryonic tissues. *PLoS One* 8:e69831
- Goodman CL, El Sayed GN, McIntosh AH, Grasela JJ, Stiles B (2001a) Establishment and characterization of insect cell lines from 10 lepidopteran species. *In Vitro Cell Dev Biol Anim* 37:367–373
- Goodman CL, McIntosh AH, El Sayed GN, Grasela JJ, Stiles B (2001b) Production of selected baculoviruses in newly established lepidopteran cell lines. *In Vitro Cell Dev Biol Anim* 37:374–379
- Hakim R, Caccia S, Loeb M, Smagghe G (2009) Primary culture of insect midgut cells. *In Vitro Cell Dev Biol Anim* 45:106–110
- Hara K, Funakoshi M, Tsuda K, Kawarabata T (1993) New *Spodoptera exigua* cell lines susceptible to *Spodoptera exigua* nuclear polyhedrosis virus. *In Vitro Cell Dev Biol Anim* 29:904–907
- Hara K, Funakoshi M, Kawarabata T (1995) A cloned cell line of *Spodoptera exigua* has a highly increased susceptibility to the *Spodoptera exigua* nuclear polyhedrosis virus. *Can J Microbiol* 41:1111–1116
- Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. *Proc Bio Sci* 270:313–321
- Hink WF, Thomsen DR, Davidson DJ, Meyer AL, Castellino FJ (1991) Expression of three recombinant proteins using baculovirus vectors in 23 insect cell lines. *Biotechnol Prog* 7:9–14
- Ishikawa H, Ikeda M, Yanagimoto K, Alves CAF, Katou Y, Lavina-Caoili BA, Kobayashi M (2003) Induction of apoptosis in an insect cell line, IPLB-Ld652Y, infected with nucleopolyhedroviruses. *J Gen Virol* 84:705–714
- Kawai Y, Mitsuhashi J (1997) An insect cell line discrimination method by RAPD-PCR. *In Vitro Cell Dev Biol Anim* 33:512–515
- Li GX, Hashimoto Y, Granados RR (2003) Growth characteristics and expression of recombinant protein by new cell clones derived from *Trichoplusia ni* (BTI Tn5B1-4) High Five cells. *Bioprocessing* 2:35–38
- Li MM, Zheng GL, Li CY (2011) Establishment and characterization of a new cell line Bm-Em-1 from *Bombyx mori* embryos. *Acta Entomol Sin* 54:1341–1347
- Liu YZ, Wei G, Lu ES, Yuan ZF (2010) The data generating model in toxicity test and estimation for the toxicity indicators. *Plant Dis Pests* 1:42–45
- Meng MJ, Li TL, Li CY, Li GX (2008) A suspended cell line from *Trichoplusia ni* (Lepidoptera): characterization and expression of recombinant proteins. *Insect Sci* 15:423–428
- Norusis MJ (2012) IBM SPSS statistics 19.0 advanced statistical procedures companion. Prentice Hall press, Upper Saddle River
- Palli SR, Caputo GF, Sohi SS, Brownwright AJ, Ladd TR, Cook BJ, Primavera M, Arif BM, Retnakaran A (1996) CfMNPV blocks AcMNPV-induced apoptosis in a continuous midgut cell line. *Virology* 222:201–213
- Saeed S, Sayyed AH, Ahmad I (2010) Effect of host plants on life-history traits of *Spodoptera exigua* (Lepidoptera: Noctuidae). *J Pest Sci* 83:165–172
- Shao HL, Zheng WW, Liu PC, Wang Q, Wang JX, Zhao XF (2008) Establishment of a new cell line from lepidopteran epidermis and hormonal regulation on the genes. *PLoS One* 3:e3127
- Si SY, Zhou LL, Wang SL, Jiang XF, Xu ZF, Mu W, Wang DS, Wang XP, Chen HT, Yang YH, Ji XC (2012) Progress in research on prevention and control of beet armyworm, *Spodoptera exigua* in China. *Chin J Appl Entomol* 49:1432–1438
- Smagghe G, Goodman CL, Stanley D (2009) Insect cell culture and applications to research and pest management. *In Vitro Cell Dev Biol Anim* 45:93–105
- Sudeep AB, Mourya DT, Shouche YS, Pidiyar V, Pant U (2002) A new cell line from the embryonic tissue of *Helicoverpa armigera* HBN (Lepidoptera: Noctuidae). *In Vitro Cell Dev Biol Anim* 38:262–264
- Sumerford DV (2003) Larval development of *Spodoptera exigua* (Lepidoptera: Noctuidae) larvae on artificial diet and cotton leaves containing a *Bacillus thuringiensis* toxin: heritable variation to tolerate Cry1Ac. *Fla Entomol* 86:295–299
- Underwood N (2011) Density dependence in insect performance within individual plants: induced resistance to *Spodoptera exigua* in tomato. *Oikos* 119:1993–1999

- Wang P, Toung R, Granados RR (1999) The establishment of new cell lines from *Pseudaletia unipuncta* with differential responses to baculovirus infection. *In Vitro Cell Dev Biol Anim* 35:333–338
- Weng QB, Yang K, Xiao W, Yuan MJ, Zhang WJ, Pang Y (2009) Establishment of an insect cell clone that harbours a partial baculoviral genome and is resistant to homologous virus infection. *J Gen Virol* 90:2871–2876
- Wu CY, Wang CH (2006) New cell lines from *Lymantria xylin*a (Lepidoptera: Lymantriidae): characterization and susceptibility to baculoviruses. *J Invertebr Pathol* 93:186–191
- Wu CY, Chen YW, Lin CC, Hsu CL, Wang CH, Lo CF (2012) A new cell line (NTU-SE) from pupal tissues of the beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), is highly susceptible to *S. exigua* multiple nucleopolyhedrovirus (SeMNPV) and *Autographa californica* MNPV (AcMNPV). *J Invertebr Pathol* 111:143–151
- Yasunaga-Aoki C, Imanishi S, Liyama K, Kawarabata T (2004) Establishment of phagocytic cell lines from larval hemocytes of the beet armyworm, *Spodoptera exigua*. *In Vitro Cell Dev Biol Anim* 40:183–186
- Zhang H, Zhang YA, Qin QL, Li X, Miao L, Wang YZ, Yang ZQ, Ding C (2006) New cell lines from larval fat bodies of *Spodoptera exigua*, characterization and susceptibility to baculoviruses (Lepidoptera: Noctuidae). *J Invertebr Pathol* 91:9–12
- Zhang H, Zhang YA, Qin Q, Li X, Miao L, Wang YZ, Qu LJ, Zhang AJ, Yang Q (2009) A cell strain cloned from *Spodoptera exigua* cell line (IOZCAS-Spex II) highly susceptible to *S. exigua* nucleopolyhedrovirus infection. *In Vitro Cell Dev Biol Anim* 45:201–204
- Zhang X, Feng Y, Ding WF, Chen XM, Wang CY, Ma T (2011) Establishment and characterization of an embryonic cell line from *Gampsocleis gratiosa* (Orthoptera: Tettigoniidae). *In Vitro Cell Dev Biol Anim* 47:327–332
- Zhang A, Li X, Zhang H, Wang H, Miao L, Zhang J, Qin Q (2012) A new cell line from *Spodoptera exigua* (Lepidoptera: Noctuidae) and its differentially expressed genes. *J Appl Entomol* 136:632–637