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SHORT COMMUNICATION

A new cell line from *Spodoptera exigua* (Lepidoptera: Noctuidae) and its differentially expressed genes

A. Zhang[1,2], X. Li[1], H. Zhang[1], H. Wang[1], L. Miao[1], J. Zhang[1] & Q. Qin[1]

1 State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China
2 Graduate University of Chinese Academy of Sciences, Beijing, China

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Correspondence
Qilian Qin, State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beichenxilu 1-5, Beijing 100101, China. E-mail: qinql@ioz.ac.cn

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Abstract

A new cell line, designated IOZCAS-Spex XI, was established from the pupal ovaries of *Spodoptera exigua* (Lepidoptera: Noctuidae) in TNM-FH medium containing 10% foetal bovine serum. The spherical cells were predominant among the various cell types. The population-doubling time during the logarithmic phase of growth was 81.7 h. It was confirmed that the cell line originated from *S. exigua* by DAF-PCR technique. Analysis of susceptibility to baculovirus showed that the new cell line was susceptible to *S. exigua* nucleopolyhedrovirus (SeNPV), *Autographa californica* multiple NPV (AcMNPV) and slightly susceptible to *S. litura* NPV (SpltNPV), while not permissive to *Helicoverpa armigera* NPV and *Hyphantria cunea* NPV (HcNPV). Real-Time PCR analysis was carried out to compare some differentially expressed genes between the cell line and the primary culture. The result showed that marked significant differences were observed in the expression of the genes of SUMO-1 activating enzyme, BCCIP-like protein, 10 kDa HSP, CypA, receptor for activated PKC, PDI-like protein ERp57, ALDH, DEAD box ATP-dependent RNA helicase-like protein ($P < 0.01$), while a significant difference was obtained in the expression of GST gene between the cell line and the primary culture ($P < 0.05$).

Introduction

Establishment of new insect cell lines often leads to increasing insect cell culture capacity for meeting various needs; especially, lepidopteran cell lines that are susceptible to infection by baculoviruses are more valuable in the fields of agriculture and biotechnology. Numerous cell lines have been previously established from lepidopteran species since the first insect cell lines were established in the early 1960s (Gaw et al. 1959; Grace 1962; Gelernter and Federici 1986; Hara et al. 1993; Granados et al. 1994). But in comparison with mammalian systems, insect cell culture is in its infancy. Cell lines from embryos, neonate larvae, larval haemocytes and larval fat bodies of the beet armyworm *Spodoptera exigua*, a major worldwide pest of economically important crops, have been reported (Gelernter and Federici 1986; Hara et al. 1993; Goodman et al. 2001; Yasunaga-Aoki et al. 2004; Zhang et al. 2006). However, no published report is available for a cell line from pupal ovaries of *S. exigua*. This report presented establishment and characterization of a new continuous cell line originated from the pupal ovaries of the insect.

Materials and Methods

Initiation and maintenance of the cell lines

The larvae of *S. exigua* were reared on an artificial diet (Li et al. 2002) at the State Key Laboratory of Integrated Pest Management, Institute of Zoology, Chinese Academy of Sciences, Beijing, China. Medium employed to initiate primary cultures was TNM-FH (Sigma, Saint Louis, USA) containing 10% foetal

bovine serum and 10% TNM-FH saturated with phenylthiourea (primary medium). The TNM-FH saturated phenylthiourea solution was omitted from the growth medium. The female pupae were collected and surface sterilized by immersing into 0.26% sodium hypochlorite solution for 5 min and then into 75% ethanol for 10 min. The ovaries were excised from the pupae, thoroughly rinsed with sterile Ringer's solution to remove haemocytes and then rinsed with the primary medium. Whole tissues were subsequently transferred to a T-25-cm^2 culture flask (Corning, NY, USA) containing 1.0 ml primary medium. The cultures were incubated at 27°C. After an attachment period (ca. 12 h), an additional 3.0 ml of the growth medium was added without disturbing the attached tissues. The primary cultures were initially fed every 7–10 days (using 2 ml medium replacement).

Characterization

Cell morphology was observed under phase contrast microscopy. The growth curve of the cell line was determined at passage 11 (Corning, NY, USA) as previously described (Zhang et al. 2006), by which cell population-doubling times were calculated (McIntosh and Ignoffo, 1989). A modified karyological analysis reported by Takahashi et al. (1980) was employed to characterize the new cell line at its 10th passage..

The cell line was identified as having originated from *S. exigua* by the gel profiles of a DNA fingerprinting-PCR (DAF-PCR) technique employing aldolase as a primer. The PCR conditions used were modified from McIntosh et al. (1996) and Liu et al. (2003) as described previously (Zhang et al. 2006). Preparation and inoculation of the viral inocula were described previously (Zhang et al. 2006). The viruses tested in these studies include *S. exigua* nucleopolyhedrovirus (SeNPV), *Autographa californica* multiple NPV (AcMNPV), *S. litura* NPV (SpltNPV), *Helicoverpa armigera* NPV (HaNPV) and *Hyphantria cunea* NPV (HcNPV).

Real-Time PCR expression analysis

Total RNAs from cells of the primary culture and IOZCAS-Spex XI were isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). After being treated with DNase I (RNase-free; TaKaRa, Dalian, China), 1 μg of RNA was reverse transcribed following the

Table 1 Primer sequences for Real-Time PCR validation of libraries

Gene	GenBank accessions	Primers (5′–3′) F: forward; R: reverse	Amplicon size (bp)
β-tubulin	EU100017.1	F: GTGGGCGGTGGTGATGACTCCTT R: GTTGTTGGCCGCATCTTCCTTACC	183
SUMO-1 activating enzyme	JK217367	F: CGC TTA TGG GGC TTG GAA TCT R: GGTGGAGCTAAAAACTGCGAATAC	182
Protein BCCIP-like protein	JK217369	F: AAGAGGCCCTGGCTAGTTTTGAC R: CCGCATGCTCCACCTCTTCT	165
Small HSP	JK217357	F: GGCTGGCCCTGACTCCTGATGA R: ACTTGTCCTTGTCGGTCCTGATGC	138
10 kDa HSP	JK217370	F: ACCCGGAGCGAGGAAAGAC R: GTGCCACCGTACTCAGGAAGG	87
3-dehydroecdysone 3 alpha-reductase	JK217359	F: CCGCAGCTCTTGTGAAATGGTC R: GTCAATATCTCTAGCGTGGTGTCC	100
GST	JK217360	F: ATACGCTGAATTCAACAAGGAT R: ACATACCCGCGAAAACAAAGTCTC	128
CypA	JK217362	F: AAAACTTCCGTGCCCTCTGC R: CAACGTGTCTGCCATCCAACC	282
Receptor for activated PKC	JK217363	F: CAAGATCCCACAAACGCAAGAGTC R: ACAGAAGCGAACCATTACGGAGTC	133
PDI-like protein ERp57	JK217366	F: GGCAAGCCCAAGAAGGAAGAG R: CTGGGATGGGATATGGACAAGTT	143
ALDH	JK217373	F: GGTGGCCCTAATGCTTGATGC R: ACAGAGATGGGTGGACCTTGAAC	112
DEAD box ATP-dependent RNA helicase-like protein	JK217374	F: AGCTCGCGGTTTACTTG R: ACTTGCACCACTGTTGTTCTC	139

protocols of SYBR® PrimeScript® RT-PCR Kit II (Perfect Real-Time) (TaKaRa). To ensure that template quantities for the reverse transcription-PCR (RT-PCR) were approximately equal, β-tubulin genes were amplified as a cDNA quantitative control. GenBank accessions, primer sequences and PCR product for all targets were listed in Table 1. The Real-Time PCR experiments were performed using Mx3000P (Stratagene, Carlsbad, CA, USA) and the data were analysed using MxPro (Stratagene). The parameters were 95°C for 30 s; followed by 40 cycles of 95°C for 5 s, 55°C for 30 s, 68°C for 15 s and a final cycle for 95°C for 1 min, 55°C for 30 s, 95°C for 30 s. The induction ratio was calculated as recommended by the manufacturer that corresponded to $2^{-\Delta\Delta CT}$, where $\Delta\Delta CT = (C_{T,Target\ gene} - C_{T,\ \beta\text{-tubulin}})_{cell\ line} - (C_{T,Target} - C_{T,\ \beta\text{-tubulin}})_{primary\ cells}$. Data were analysed with standard one-way analysis of variance (ANOVA), using the SPSS statistical software.

Results and Discussion

Primary cultures and subcultures

Successful cultures were initiated in June 2010. Three days after initiation, the pupal ovary tissues adhered tightly to the flask bottom, around which numerous cells attached, detached and spread out (Fig. 1a). Nine days later, the cells approached confluence. The first subculture was performed in a month later by transferring the contents of the culture flask to a new one containing 2 ml fresh growth medium. From the initial subculture to the 10th passage, the interval between subcultures ranged from 7 to 8 days, depending on the growth rate of the cells (Fig. 1b). After the 10th passage, the cells propagated rapidly, and thereafter the interval between passages was 4–5 days. Up to now, the cell line, designated IOZCAS-Spex XI, has been subcultured for more than 30 generations.

Characterization

The cell cultures were heterogeneous in shape, consisting of spherical, spindle-shaped and macrophage-like cells. The population-doubling time during the logarithmic phase of growth was 81.7 h. Chromosome studies of the cell line showed a typical lepidopteran pattern (Hara et al. 1993) (Fig. 2).

The DAF-PCR gel profiles of the cell line, its host and other originations conformed that the cell line originated from *S. exigua*. The new cell line had similar patterns with its insect host (*S. exigua* ovary) and another same originating cell line IOZCAS-Spex II-A, a cell clone from larval fat bodies of *S. exigua* (Zhang et al. 2009), while it had distinctive patterns from that of other two cell lines maintained in this laboratory (Sf9, a cell line from *S. frugiperda*, Vaughn et al. 1977; BCIRL-Hz-AM1, a cell line from *H. zea*, McIntosh et al. 1983) (Fig. 3).

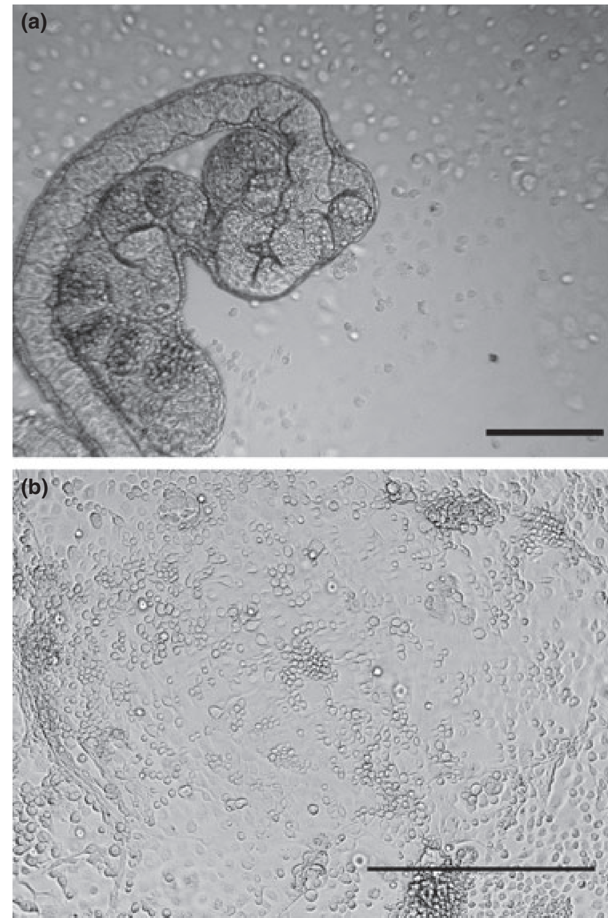

Fig. 1 (a) Cell migration occurred within 48 h after the culture was set up. The bar is 200 μm. (b) Monolayer of the cultured cell (IOZCAS-Spex XI) at passage 10. The bar is 400 μm.

Typical cytopathogenic effects were observed in the cell line when it was inoculated with either SeNPV, AcMNPV or SpltNPV, while it was not susceptible to the infection of HaNPV and HcNPV. At 7 days pi approximate, 80% of IOZCAS-Spex XI cells were infected with SeNPV and approximate 90% infection was observed in the AcMNPV inoculation (Fig. 4a,b). Interestingly, the new cell line was also susceptible to the infection of SpltNPV, an NPV not infecting *S. exigua*; though, the per cent infection was only 27% (Fig. 4c). This result implies that the cell line may be applied to the research of SpltNPV, for which

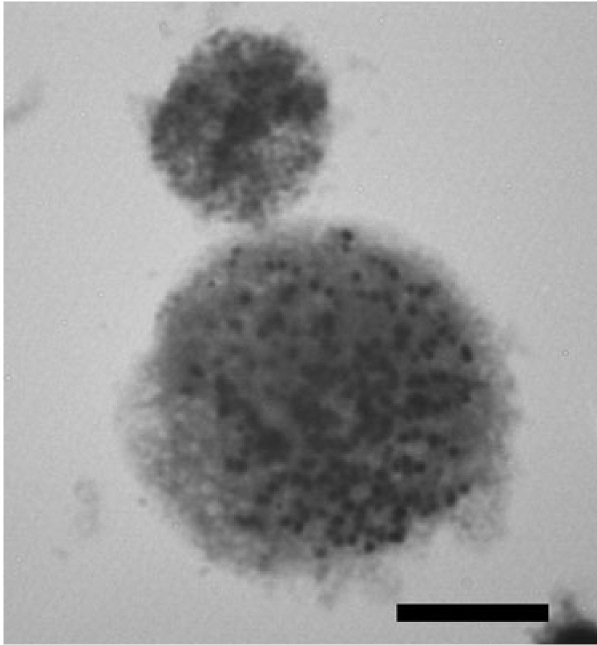

Fig. 2 A typical chromosome spread from IOZCAS-Spex XI at passage 10. Marker bar is 100 μm.

a relative limited cell lines are able to be available (Jen and Lin 1995; Mitsuhashi 1995; Shih et al. 1997; Pant et al. 1998, 2000; Paul and Ramakrishnan 2000). Further work to clone the IOZCAS-Spex XI cell line should be performed to attempt to gener-

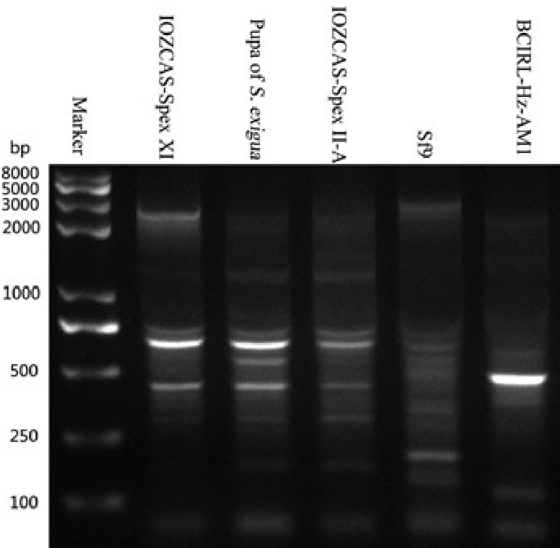

Fig. 3 DAF-PCR profiles of the new established cell line IOZCAS-Spex XI, its origination and other cell lines in our laboratory: Sf9, BCRIL-HzAM1, IOZCAS-Spex II-A (a cloned cell strain originated from *S. exigua*). Major bands identified are shared between the cell lines and their host, while the other cell lines show different profiles.

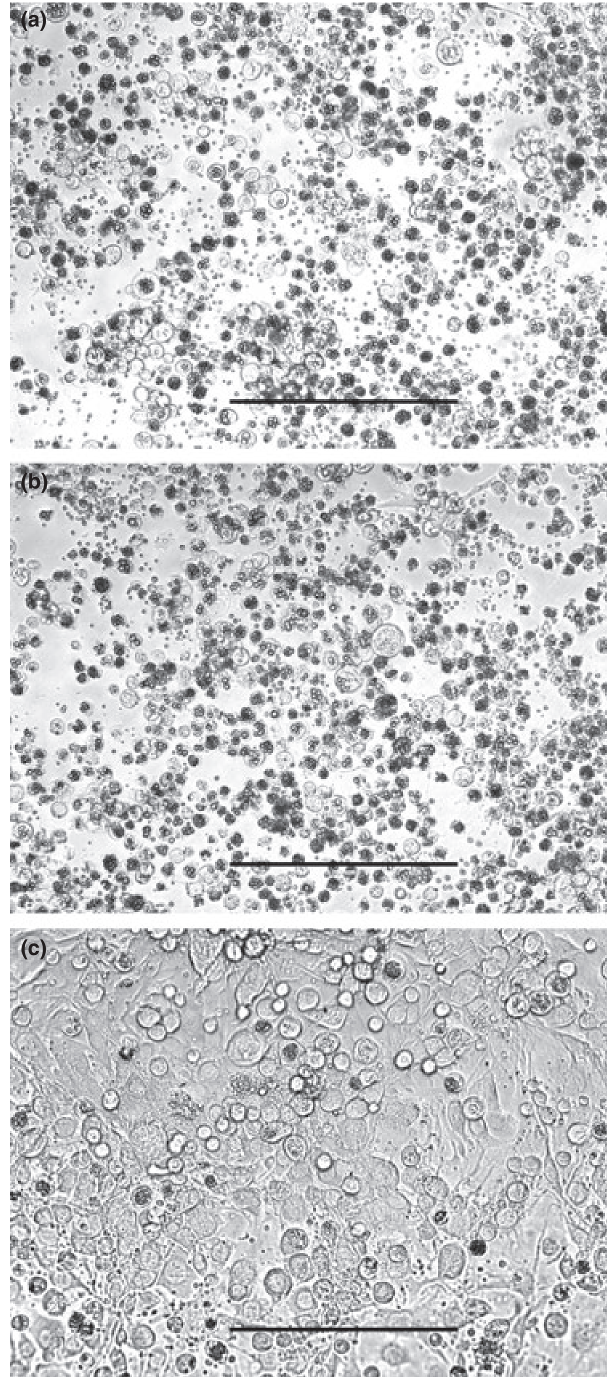

Fig. 4 (a) Majority of cells infected with SeNPV showing the cell line to be susceptible to the infection of the baculovirus. The bar is 200 μm. (b) Majority of cells infected with AcMNPV showing the cell line to be susceptible to the infection of the baculovirus. The bar is 200 μm. (c) Infection of cells by SpltNPV showing a slight susceptibility to infection The bar is 200 μm.

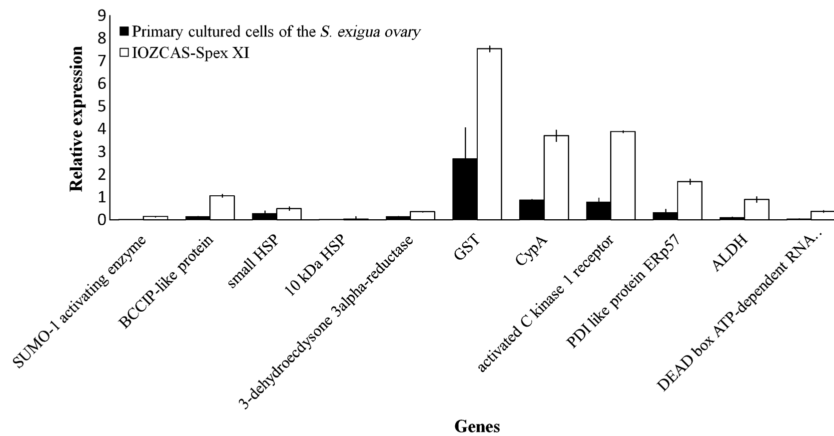

Fig. 5 Real-Time PCR analyses for the complement component SUMO-1 activating enzyme, BCCIP-like protein, small HSP, 10 kDa HSP, 3-dehydroecdysone 3 alpha-reductase, GST, CypA, receptor for activated PKC, PDI-like protein ERp57, ALDH, DEAD box ATP-dependent RNA helicase-like protein, with β-tubulin as a cDNA quantitative control. Error bars indicated standard errors for Real-Time PCR.

ate a cell strain that is more susceptible to SpltNPV. Production of occlusion bodies (OBs) of IOZCAS-Spex XI infected with SeNPV and AcMNPV was 8.2 $\pm$ 1.5 OBs/cell and 5.3 $\pm$ 1.3 OBs/cell, respectively.

Relative quantification of differentially expressed genes

A previous study using suppression subtractive hybridization to compare differentially expressed genes between the cell line and its parental cells had screened several critical genes that needed further analysis (data not shown). In the present study, we randomly selected 11 genes to carry out Real-Time PCR analysis to compare gene expression between the primary cell culture and resulting cell line. There were marked significant differences in the expression of genes of SUMO-1 activating enzyme, BCCIP-like protein, 10 kDa HSP, CypA, receptor for activated PKC, PDI-like protein ERp57, ALDH, DEAD box ATP-dependent RNA helicase-like protein, between cell line and its primary culture ($P < 0.01$), and that of GST showed significant difference ($P < 0.05$). The expression of genes of small HSP and 3-dehydroecdysone 3 alpha-reductase was confirmed to be not significant by statistical analysis (Fig. 5). The differentially expressed genes between the cell line and its primary cell culture implied that they might play important roles in the transformation of the primary cultured *S. exigua* ovary cells to the continuous cell line.

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