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ORIGINAL ARTICLE

Basic Study

Expression of hepatitis B virus surface antigens induces defective gonad phenotypes in *Caenorhabditis elegans*

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Abstract

AIM

To test whether a simple animal, *Caenorhabditis elegans* (*C. elegans*), can be used as an alternative model to study the interaction between hepatitis B virus antigens (HBsAg) and host factors.

METHODS

Three plasmids that were able to express the large, middle and small forms of HBsAgs (LHBsAg, MHBsAg, and SHBsAg, respectively) driven by a ubiquitous promoter (*fib-1*) and three that were able to express SHBsAg driven by different tissue-specific promoters were constructed and microinjected into worms. The brood size, egg-laying rate, and gonad development of transgenic worms were analyzed using microscopy. Levels of mRNA related to endoplasmic reticulum stress, *enpl-1*, *hsp-4*, *pdi-3* and *xbp-1*, were determined using reverse transcription polymerase reaction (RT-PCRs) in three lines of transgenic worms and dithiothreitol (DTT)-treated wild-type worms.

RESULTS

Severe defects in egg-laying, decreases in brood size, and gonad retardation were observed in transgenic worms expressing SHBsAg whereas moderate defects were observed in transgenic worms expressing LHBsAg and MHBsAg. RT-PCR analysis revealed that *enpl-1*, *hsp-4* and *pdi-3* transcripts were significantly elevated in worms expressing LHBsAg and MHBsAg and in wild-

type worms pretreated with DTT. By contrast, only *pdi-3* was increased in worms expressing SHBsAg. To further determine which tissue expressing SHBsAg could induce gonad retardation, we substituted the *fib-1* promoter with three tissue-specific promoters (*myo-2* for the pharynx, *est-1* for the intestines and *mec-7* for the neurons) and generated corresponding transgenic animals. Moderate defective phenotypes were observed in worms expressing SHBsAg in the pharynx and intestines but not in worms expressing SHBsAg in the neurons, suggesting that the secreted SHBsAg may trigger a cross-talk signal between the digestive track and the gonad resulting in defective phenotypes.

CONCLUSION

Ectopic expression of three forms of HBsAg that causes recognizable phenotypes in transgenic worms suggests that *C. elegans* can be used as an alternative model for studying virus-host interactions because the resulting phenotype is easily detected through microscopy.

Key words: Hepatitis B virus; *Caenorhabditis elegans*; Green fluorescence proteins; Endoplasmic reticulum stress; Gonad retardation; Surface antigens

Core tip: In the past, mouse and cell culture models have been used for studying the effects of hepatitis B virus antigens (HBsAg) on hosts. Both models have advantages and disadvantages in terms of economic and time concerns. In this study, we provide an alternative animal model, *Caenorhabditis elegans (C. elegans)*, to demonstrate that SHBsAg can induce observable phenotypes which has never been reported in mouse and cell culture models. We suggest that *C. elegans* can serve as a new plateform for studying various viral pathogenesis.

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INTRODUCTION

Human hepatitis B virus (HBV), a member of the family *Hepadnaviride*, is a partially double-stranded DNA virus. The genome of HBV contains approximately 3200 nucleotides that encodes four open reading frames, namely surface (S), core (C), polymerase (P) and X in an overlapping but frame-shifted manner[1-3]. Infection with HBV inducers a broad range of clinical outcomes, from asymptomatic hepatitis to fulminant hepatitis. Chronic hepatitis B carriers are highly associated with the development of liver cirrhosis and hepatocellular carcinoma[4,5]. Molecular biology analyses from tumor samples have revealed that HBV DNA integration could activate genes associated with the cell cycles, leading to abnormal cell proliferation[6,7]. Pathogenesis and etiology studies have found that X and truncated preS proteins play oncogenic roles[8-10].

Woodchucks were the first animal model for studying liver carcinogenesis caused by natural woodchuck hepatitis virus (WHV) infection[11,12]. A high incidence of liver tumor formation occurs in newborn woodchucks infected with WHV. Molecular dissection has revealed that *c-myc* oncogene level are highly elevated in liver tumors[13,14]. Later, the transgenic mouse model was applied to express an individual viral protein, such as the large hepatitis B surface antigen (LHBsAg) and X protein, which are driven by the albumin promoter for specific expression in the liver, to study the mechanisms of liver carcinogenesis induced by viral proteins[15,16]. In combining molecular biology analyses of the HBV X (HBx) gene in transfected cells, numerous studies have elucidated that the X protein is multifunctional and induces transactivation activity, signal transduction and cell death[9,17,18]. Recently, Geng *et al*[19] employed *Caenorhabditis elegans* (*C. elegans*), a soil nematode, to express HBx under a heat shock control and found that HBx induced cell apoptosis and necrosis through the interaction of HBx and CED-9, a human homolog of Bcl-2.

C. elegans was first used as a model organism for studying development and the nervous system because the species is transparent throughout its life span and in its adult form, possesses approximately 300 neurons out of 1000 somatic cells[20,21]. Because of its short-life cycle, simplicity, numerous available mutated forms, and ease of handling for knocking-down specific gene, *C. elegans* is now a model for studying various biological topics, such as aging, human diseases, host-pathogen interaction and viral pathogenesis[22-25]. Because of a high percentage of genes in numerous cellular pathways is conserved across nematodes to vertebrates, a study of PEG-mediated *Poxviridae* infection in *C. elegans* revealed that the core genes of apoptosis (*ced-3* and *ced-4*) control vaccinia virus replication in worms[26,27]. Therefore, *C. elegans* could serve as a new platform for virologists to study virus-host interaction and pathogenesis in addition to the currently used cell culture and mammalian models. In this study, we expressed three forms of HBsAg in *C. elegans* to determine different degrees of defects in gonad development.

MATERIALS AND METHODS

Plasmid constructions

$P_{fib-1::}$gfp::icr::SHBsAg: A 1.5 kb fragment excised from the $P_{fib-1::}$gfp::LD plasmid[28] by cutting with *Hind*Ⅲ and *Age* Ⅰ and was isolated and then inserted into the *Hind*Ⅲ and *Age* Ⅰ sites of pPD95.75 to generate $P_{fib-1::}$gfp. P_{fib-1}::gfp was then cut with *EcoR* Ⅰ and ligated with a 0.8 kb of icr::SHBsAg fragment which was isolated from $P_{fib-1::}$LD::icr::SHBsAg to generate a 6.8 kb of $P_{fib-1::}$

gfp::icr::SHBsAg. When microinjection of $P_{fib-1::}$gfp::icr::SHBsAg into N2 strain, worms expressed green fluorescence proteins (GFP) and HBV small surface antigens (SHBsAg).

$P_{fib-1::}$gfp::icr::linker: A linker was designed to contain *EcoR* Ⅰ, *Not* Ⅰ, *Bgl* Ⅱ, *Sal* Ⅰ, *Nsi* Ⅰ and *Sac* Ⅰ cutting sites. This linker was ligated to $P_{fib-1::}$gfp and generated plasmid $P_{fib-1::}$gfp::linker. $P_{fib-1::}$gfp::linker was cut with *EcoR* Ⅰ *and Not* Ⅰ and ligated with an icr fragment which was isolated from $P_{fib-1::}$gfp::icr::SHBsAg to generate a 6.2 kb of $P_{fib-1::}$gfp::icr::linker plasmid.

$P_{fib-1::}$gfp::icr::MHBsAg: A 680 bp of MHBsAg DNA fragment was amplified from pMH3/3097[29] using primers HBVs(M)-Not Ⅰ-F and HBVs-Sal Ⅰ-R. This fragment was ligated with $P_{fib-1::}$gfp::icr::linker to generate a 7.0 kb of $P_{fib-1::}$gfp::icr::MHBsAg. Transgenic worm carrying $P_{fib-1::}$gfp::icr::MHBsAg expressed GFP and MHBsAg.

$P_{fib-1::}$gfp::icr::LHBsAg: A 1.1 kb of LHBsAg DNA fragment was amplified from pMH3/3097[29] using primers HBVs(L)-NotI-F and HBVs-Sal Ⅰ-R. This fragment was ligated with $P_{fib-1::}$gfp::icr::linker to generate a 7.3 kb of $P_{fib-1::}$gfp::icr::LHBsAg. When microinjection of $P_{fib-1::}$gfp::icr::LHBsAg into N2 strain, worms expressed GFP and LHBsAg.

$P_{myo-2::}$gfp::icr::SHBsAg: The fragment of *fib-1* promoter was cut from P_{fib-1}GFP-icr-SHBsAg by digestion with *Hind* Ⅲ and *Age*I, and replaced with the *myo-2* promoter which was isolated from $P_{myo-2::}$gfp::icr::DsRed::LD[28] to create a 6.6 kb of $P_{myo-2::}$gfp::icr::SHBsAg. Transgenic worms carrying this plasmid expressed both GFP and SHBsAg in pharynx.

$P_{mec-7::}$gfp::icr::SHBsAg: The plasmid was generated by substitution of the *fib-1* promoter of $P_{fib-1::}$gfp::icr::SHBsAg with the *mec-7* promoter which was isolated from $P_{mec-7::}$gfp::icr::DsRed::LD[28] to create a 6.3 kb of $P_{mec-7::}$GFP::icr::SHBsAg. Transgenic worms carrying this plasmid expressed GFP and SHBsAg in neurons.

$P_{ges-1::}$gfp::icr::SHBsAg: The plasmid was generated by substitution of the *fib-1* promoter of $P_{fib-1::}$gfp::icr::SHBsAg with the *ges-1* promoter which was isolated from $P_{ges-1::}$gfp::icr::DsRed::LD[28] to create a 6.6 kb of $P_{ges-1::}$gfp::icr::SHBsAg. Transgenic worms carrying this plasmid expressed GFP and SHBsAg in intestinal cells.

Primers used in this study

For plasmid constructions and RT-PCR analyses, the following paired primers were used: linker-F: 5′-aat tcaaaaagcggccgcagatctgtcgacatgcatgagctc-3′; linker-R: 5′-gtttttcgccggcgtctagacagctgtacg tactcgagttaa-3′; HBVs(L)+Not Ⅰ-F: 5′-gggaacaagag cggccgcatggggcag-3′; HBVs(M)+Not Ⅰ-F: 5′-acactc atcgcggccgcatgcagtgg-3′; HBVs+Sal Ⅰ-R: 5′-gttttgtgtcgacttaaatgtataccc-3′; eft-2-F: 5′-ggtggtcaaatcatcccaac-3′; eft-2-R: 5′-tcc-tcgaaaacgtgtcctct-3′; endoplasmin-F: 5′-t gaaaa cctccaacagcaca-3′; endoplasmin-R: 5′-gcagtttccttg agccagtc-3′; hsp-4-F: 5′-ttttcgaggttcttgccact-3′; hsp-4-R: 5′-tctccggtattttcgacacc-3′; PDI-F: 5′-gccgtttcca aca aagaaga-3′; PDI-R: 5′-cccttgagc ccatcagtaga-3′; xbp-1-F: 5′-cgtcgtcta cgaagaagaagtcgtc-3′; xbp-1-R: 5′-gatg ata gttagatacatatccacactg-3′.

Worm strains and culture

N2 (wild-type) worm was obtained from the *Caenorhabditis* Genetics Center (CGC, University of Minnesota) and cultured on Nematode Growth Medium (NGM) following standard methods[20]. Images of transgenic worms were acquired using Leica DM2500 equipped with CoolSNAP K4 (photometrics) and processed with a MetaMorph (version 6.1).

Microinjection

Plasmid DNA was prepared by using QIAprep spin miniprep kit and the concentration was adjusted at 100 ng/μL in injection buffer (20 mmol/L potassium phosphate, pH 7.5, 3 mmol/L potassium citrate, pH 7.5, 2% polyethylene glycol, M.W. 6000). The injection mixture also contained pRF-4 which was included as a screening marker. Worm was placed onto 2% agarose pads and injected by capillary needle loaded with DNA mixture using a FemtoJet system (Eppendorf AG, Hamburg, Germany). The glass capillaries were purchased from World Precision Instruments (Kwik-Fil™, borosilicate 16 glass capillaries, item number 1B100F-6, United States) and pull by Flaming/Brown micropipette puller (MODEL P-97, Sutter Instrument Co., United States).

Measurement of egg-laying activity and brood size

Worms were first synchronized and placed one worm per a single plate. The offspring in each plate were counted every day.

Microscopy

For visualization of GFP expression in transgenic worms, an upright fluorescence microscope (Leica DM2500) was used. For visualization of gonad structure and development a differential interference contrast (DIC) microscope was used and images were captured using a cool CCD (CoolSNAP K4).

Reverse transcription

The total RNA was extracted from transgenic worms expressed both GFP and HBsAgs with TRIzol reagent. The reverse transcription reaction was first carried out with 4 μg of RNA, 2 μL of dNTP (10 mmol/L), 2 μL of Oligo-dT (10 mmol/L), and added DEPC H_2O to 12 μL. After incubated at 68 ℃ for 5 min, the mixture was then added 4 μL of 5 × first-strand buffer (invitrogen), 2 μL of DTT (0.1 mmol/L, invitrogen), 1 μL of RNase inhibitor (invitrogen) and 1 μL of Reverse Transcriptase (invitrogen) and incubated at 42 ℃ for 50 min, and then 70 ℃ for

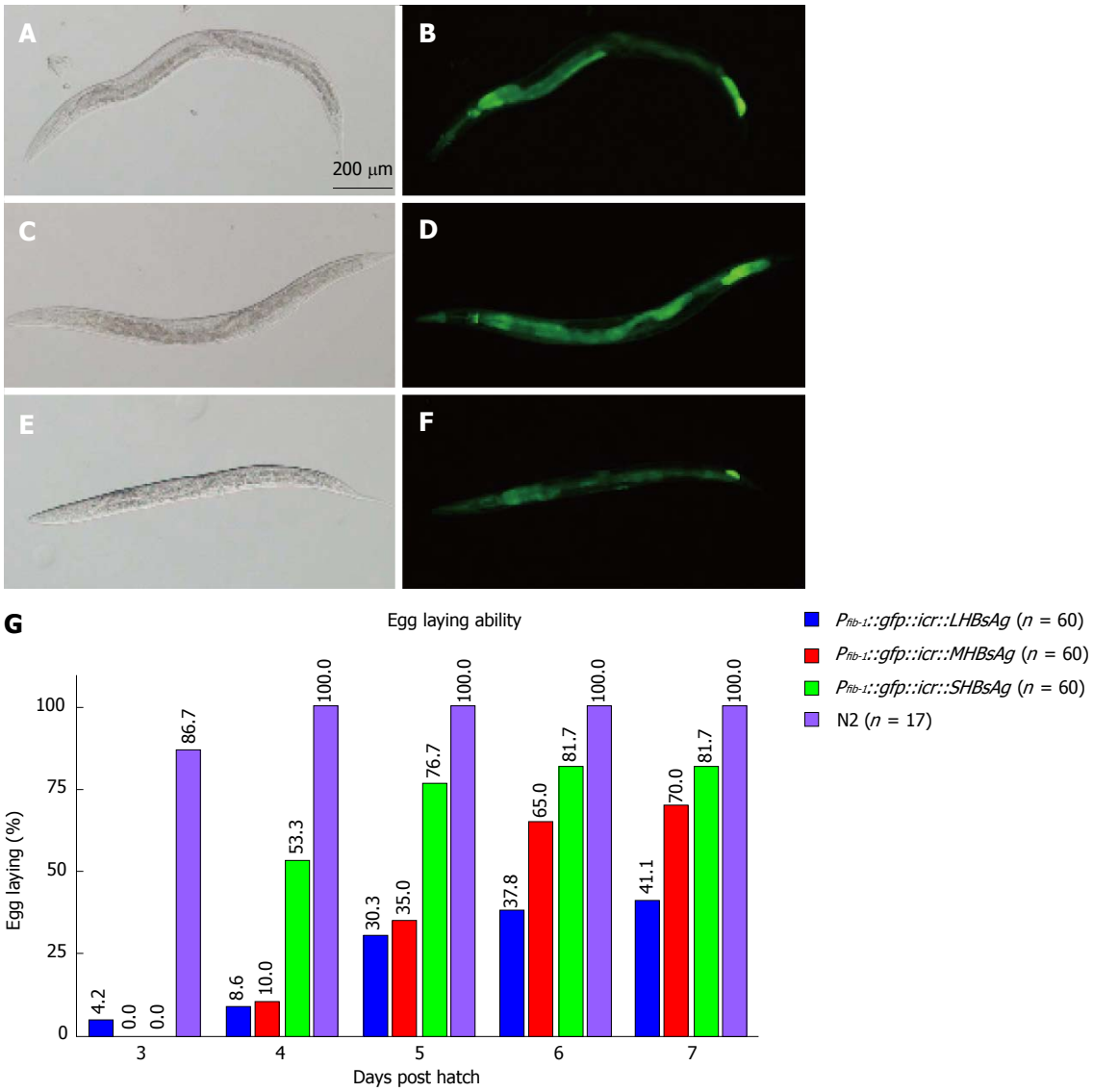

Figure 1 Expression of various lengths of hepatitis B virus antigens in whole worms induced defects in the rate of egg-laying. A-F: Micrographs of transgenic worms expressing LHBsAg (A and B), MHBsAg (C and D), and SHBsAg (E and F) were captured under a bright-field microscope (A, C, and E) and a fluorescence microscope (B, D, and F). The heads of the worms are shown toward the left. The scale bar indicates 200 μm. G: Egg-laying capability of three lines of transgenic worms and wild-type worms (N2) shown using various color bars. The rate of egg-laying in 3 to 7 d post-hatching is shown above the bar. HBsAgs: Hepatitis B virus antigens.

15 min. The primers used in PCR analyses were listed as above.

RESULTS

Expression of three forms of HBsAg reduces egg-laying capability

To determine whether *C. elegans* can be a new platform for studying virus-host interaction we ectopically expressed three lengths of HBsAg (SHBsAg, MHBsAg, and LHBsAg) in worms under the control of the ubiquitous promoter fibrillarin (*fib-1*). Three HBsAg gene sequences were individually placed in a bicistronic vector behind a reporter gene, green fluorescence protein (GFP), which was used as a selection marker[30]. Transgenic worms were selected for the expression of GFP (Figure 1A-F) and maintained to characterize phenotypes. After synchronization, transgenic worms were singled out and placed on single plates, and the numbers of eggs produced by individual worms were counted every day. The results showed different egg-laying averages in a total of 60 transgenic animals in three groups expressing SHBsAg, MHBsAg, and LHBsAg (Figure 1G). On the fourth day after hatching, the wild-type worms (N2) displayed an egg-laying rate of 100% whereas those worms expressing SHBsAg, MHBsAg, and LHBsAg demonstrated laying-egg rates of approximately 8.6%, 10%, and 53.3%, respectively. Although the egg-laying rates of the three lines of transgenic worms increased in the following days, the maximum egg-laying rate was 41.1% for worms expressing SHBsAg, 70% for worms expressing MHBsAg and 81.7% for worms expressing LHBsAg at 7 d post-hatching (Figure 1B). The reduced rate of egg-laying in the three lines of HBsAg-expressing

Table 1 Comparison of egg-laying ability and brood size among various transgenic worms

Construct	Strain	Ecotopic proteins	Protein expression site	Egg-laying ability (%)	Brood size
	N2			100 (n = 17)	290 ± 15 (n = 17)
Pfib-1::gfp::icr	N2	GFP	Whole worm	100 (n = 10)	268 ± 29 (n = 18)
Pfib-1::gfp::icr::SHBsAg	N2	GFP, SHBsAg	Whole worm	9 (n = 60)	66 ± 15 (n = 14)
Pfib-1::gfp::icr::MHBsAg	N2	GFP, MHBsAg	Whole worm	10 (n = 60)	175 ± 50 (n = 15)
Pfib-1::gfp::icr::LHBsAg	N2	GFP, LHBsAg	Whole worm	54 (n = 60)	239 ± 14 (n = 15)
Pmyo-2::gfp::icr::SHBsAg	N2	GFP, SHBsAg	Pharynx	83 (n = 60)	163 ± 20 (n = 26)
Pges-1::gfp::icr::SHBsAg	N2	GFP, SHBsAg	Intestine	97 (n = 60)	203 ± 50 (n = 32)
Pmec-7::gfp::icr::SHBsAg	N2	GFP, SHBsAg	Neuron	100 (n = 60)	270 ± 42 (n = 26)

HBsAgs: Hepatitis B virus antigens; GFP: Green fluorescence proteins; SHBsAg: Human hepatitis B virus small surface antigens; MHBsAg: Human hepatitis B virus middle surface antigens; LHBsAg: Human hepatitis B virus large surface antigens.

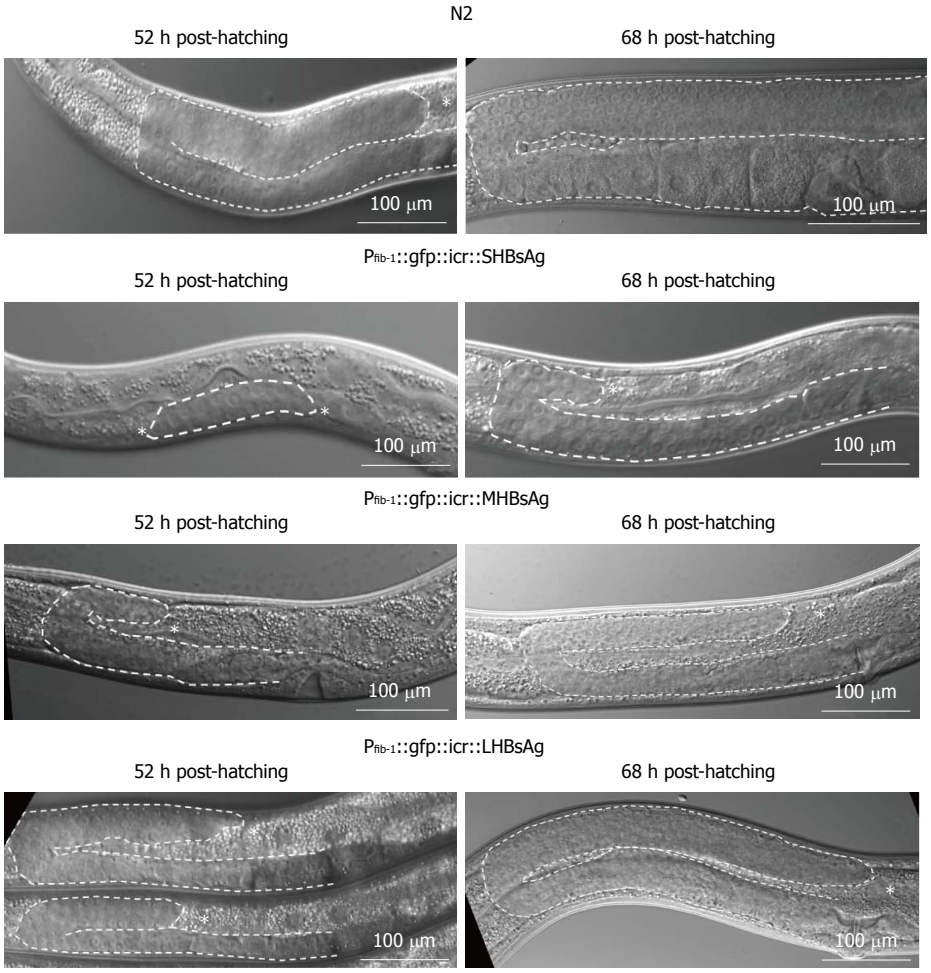

Figure 2 Differential interference contrast micrographs of gonad development in transgenic worms at various post-hatching times. The upper row shows a wild-type worm (N2); the second row shows a transgenic worm expressing SHBsAg; the third row shows a transgenic worm expressing MHBsAg; and the bottom row shows a transgenic worm expressing LHBsAg. The right column shows the gonads at 52 h post-hatching and the left column at shows gonads at 68 h post-hatching expect the worm expressing SHBsAg is at 72 h post-hatching. The gonad contour is indicated with dotted lines and the tip (distal end) is marked by asterisk.

worms was unlikely to have caused by the ectopic expression of GFP because worms carrying a plasmid with the sole function of expressing GFP throughout the body exhibited egg-laying capability of 100% (Table 1).

Expression of SHBsAg causes the most severe gonad retardation

To understand why the expression of various lengths of HBsAg in transgenic worms caused a reduction in egg-laying capability, we examined the gonad development of the three types of transgenic worms under a DIC microscope. As shown in Figure 2 (upper two rows), at 52 h after hatching, wild-type worms had nearly completed the gonad development; by contrast, worms expressing SHBsAg exhibited a dramatic retardation of gonad development in larval stage 3 (L3). The process of oogenesis was observed in N2 worms 68 h post hatching whereas the gonads of worms expressing SHBsAg were

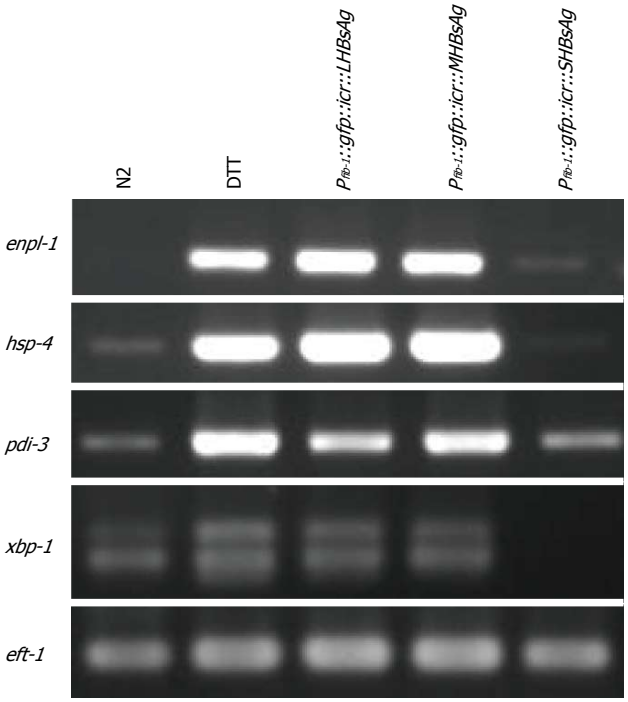

Figure 3 Reverse transcription polymerase reaction analyses of mRNA levels in various transgenic worms. The four transcripts (*enpl-1*, *hsp-4*, *pdi-3*, and *xbp-1*), ER-stress markers, from wild-type worms (N2) with or without DTT pretreatment, an ER stress inducer, and three lines of transgenic worms were analyzed using RT-PCR and gel-electrophoresis. The transcript of the translation factor (*eft-2*) served as a loading control. N2 worms treated with and without DTT and transgenic worms are indicated above the gel. DTT-treated worms severed as positive controls of ER-stress responses. DTT: Dithiothreitol; RT-PCR: Reverse transcription polymerase reaction.

only just beginning to turn as mid-stage of larva 4 as 72 h post-hatching. Oogenesis was observed in some worms expressing SHBsAg until 96 h post-hatching (data not shown). Worms expressing MHBsAg and LHBsAg at 52 h and 68 h post-hatching showed a retardation of gonad development that was less severe than that observed in worms expressing SHBsAg at a similar stage (Figure 2, lower two rows). The severity of gonad retardation clearly reflected the reduced percentage of egg-laying (9%, 10%, and 54%, respectively) and average brood size (66, 175, and 239, respectively) in the three lines of transgenic worms, as shown in Table 1.

Gonad retardation caused by SHBsAg may operate through unknown pathways

A previous study reported that endoplasmic reticulum (ER) stress could cause retardation of gonad development[31]. To determine whether the defective phenotypes of the three lines of transgenic worms resulted from ER stress, we performed RT-PCR analysis of ER stress markers. Total RNA from the three lines of transgenic worms and N2 worms with or without DTT treatment was isolated and analyzed for the expression levels of *enpl-1*, *hsp-4*, *pdi-3* and *xbp-1* through RT-PCR. The results of the gel-electrophoresis of RT-PCR products indicated that the levels of *enpl-1, hsp-4* and *pdi-3* were substantially elevated in worms expressing LHBsAg and MHBsAg, being similar to those N2 worms pretreated with the ER stress-inducer DTT whereas the *xbp-1* level had increased only slightly (Figure 3). Only a slight increase in the level of *pdi-3* was observed in worms expressing SHBsAg compared with that of wild-type worms, and no obvious elevation of other ER stress-related transcripts was detected. We concluded that the defective phenotypes caused by the expression of LHBsAg and MHBsAg were likely attributable to ER stress signals. By contrast, the defect induced by the expression of SHBsAg might have been caused by other unknown pathways.

Expression of SHBsAg in the pharynx and intestines induces defective phenotypes

To determine which tissues expressing SHBsAg were responsible for gonad retardation, we substituted the *fib-1* promoter with *myo-2*, *ges-1*, and *mec-7*, to express SHBsAg in the pharynx, intestines, and neurons, respectively. Transgenic worms expressing GFP in the pharynx, intestines, and neurons were selected (Figure 4A) and analyzed for egg-laying capability and gonad development. As shown in Table 1, 100% and 97% egg-laying capability were found in worms expressing SHBsAg in the neurons and intestines, respectively, compared with 83% egg-laying capability in worms expressing SHBsAg through *myo-2*. When examining the gonad development of the three lines of transgenic worms using DIC, we found no obvious retardation in worms expressing SHBsAg in neuron at 52 h and 68 h post-hatching and a moderate level of gonad retardation in worms expressing SHBsAg in the pharynx and intestines (Figure 4B). This suggested that cross-talk between the digestive and reproductive system may be triggered by secreted SHBsAg, causing defects in a small portion of the population.

DISCUSSION

In this study, we demonstrated that transgenic worms expressing three forms of HBsAgs throughout the body exhibited lower rates of egg-laying, reduced brood sizes and retardation of gonad development to various degrees. Unexpectedly, worms expressing SHBsAg displayed the most severe defects (Table 1). No study has yet reported that the expression of SHBsAg can induce detectable phenotypes in cultured cells or animals; however, ER-stress and tumor formation have been observed in cells and animals expressing LHBsAg and MHBsAg[16,32]. Consistent with previous studies, worms expressing LHBsAg and MHBsAg were found to possess higher levels of *enpl-1*, *hsp-4*, *pdi-3* and *xbp-1* transcripts as did N2 worms pretreated with DTT (Figure 3). Because the gonad is the organ most sensitive to environmental changes[33], we suggest that ER-stress signal occurring autonomously or non-autonomously in the gonad can lead to gonad retardation, a reduced rate of egg-laying, and a smaller brood sizes in transgenic worms expressing LHBsAg and MHBsAg.

The unexpected results of the most severe pheno-

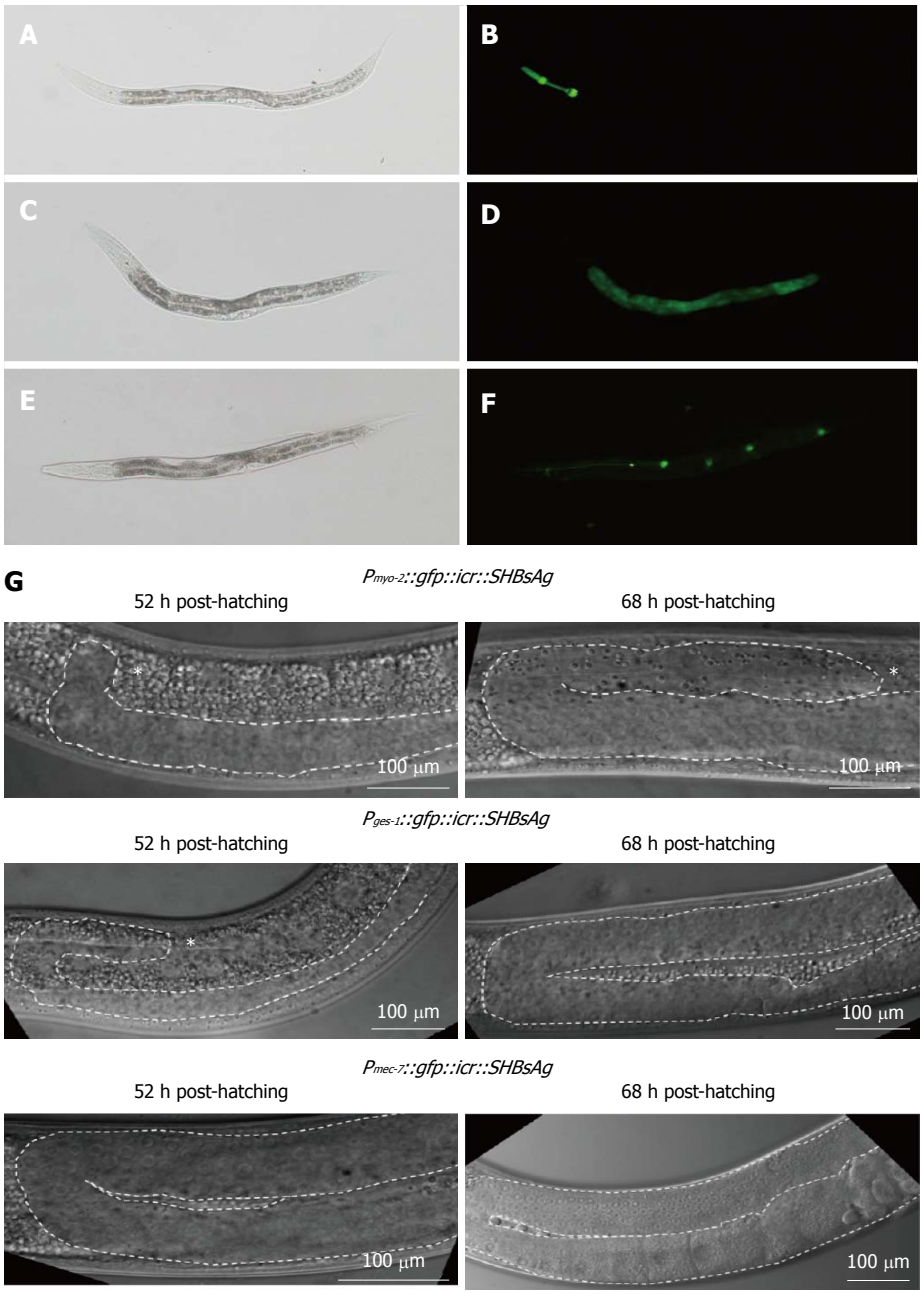

Figure 4 Features of transgenic worms expressing SHBsAgs in different tissues. A-F: Micrographs of transgenic worms expressing SHBsAgs in the pharynx (A, and B), intestinal cells (C and D), and neurons (E and F) were captured under a bright-field microscope (A, C, and E) and a fluorescence microscope (B, D, and F). The heads of the worms are shown toward the left. The scale bar indicates 200 μm; G: Gonad development in transgenic worms: The upper row is a transgenic worm expressing SHBsAg in the pharynx; the middle row is a transgenic worm expressing SHBsAg in intestinal cells; and the lower row is a transgenic worm expressing SHBsAg in neurons. Images were captured under a DIC microscope. The right column shows the gonads at 52 h post-hatching and the left column at 68 h post-hatching. The gonad contour is outlined by dotted lines and the tip (distal end) is marked by asterisk. HBsAgs: Hepatitis B virus antigens.

types induced by the expression of SHBsAg might be explained by the different nature of the three forms of HBsAg. In general, SHBsAg can form subviral particles of approximately 22 nm and be constantly secreted outside of cells whereas MHBsAg is less efficiently secreted and LHBsAg is usually retained in the ER[29,32]. This hypothesis is supported by the results shown in Figure 3, namely that four ER-stress related transcripts (*enpl-1*, *hsp-4*, *pdi-3* and *xbp-1*) were substantially elevated in worms expressing LHBsAg and MHBsAg but only one transcript (*pdi-3*) displayed a slight elevation in worms expressing SHBsAg. The secretion of SHBsAg might either trigger signals inhibiting gonad development or titrate out secretion factors that are required for gonad development, although ER-stress signals might also play a minor role (Figure 4). Nevertheless, the underlying mechanism that leads to the most severe phenotypes in worms expressing SHBsAg remains unknown and will be elucidated by performing rescue and genetic cross experiments in the future.

C. elegans has been used for studying viral pathogenesis and virus-host interaction for more than a

decade[27,34]. In comparison with the number of publications using *C. elegans* study viral, bacterial and fungal pathogenesis, relatively few papers have focused on viral pathogenesis and virus-host interaction in the past 10 years. The bottleneck could be due to the difficulty of creating transgenic worms expressing viral antigens. Currently, two methods for delivering ectopic genes into *C. elegans* are microinjection and gene bombardment, neither of which are easily achievable in general biology laboratories. To use *C. elegans* as a platform for studying virus-host interaction, virologists must collaborate with worm scientists. Alternatively, virologists could engineer the three viruses (Orsay, Santeui, and Le Blanc virus[35,36]), that naturally infect *C. elegans* to become versatile vectors for the easy deliver of different viral genes into worms through infection.

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COMMENTS

Background

The purpose of this research was to develop a model for human hepatitis B viral antigen interaction with host factors in the standard model animal *Caenorhabditis elegans* (*C. elegans*).

Research frontiers

The hepatitis B viral surface proteins (large, small and middle sized) (LHBsAg, MHBsAg, SHBsAg) are essential in viral assembly and infection. Here the three viral surface proteins were synthesized in *C. elegans* after microinjection of bacterial plasmids containing the genes for the proteins expressed under the control of a ubiquitous animal promoter. Severe reduction in egg laying and brood size as well as gonad retardation occurred with expression of the small hepatitis B virus (HBV) surface antigen in the worm. Smaller effects were found with the middle and larger sized surface antigens.

Innovations and breakthroughs

The specific effects of human HBV surface antigen expression of the *C. elegans* worm demonstrates the worm as a useful model for understanding viral infection and its effects on animal tissues.

Applications

Simple and rapid animal cell and model animal systems are essential for understanding of the infection process and disruptions caused by important human disease agents such as HBV. Here a new and useful model is established and its characteristics presented.

Terminology

HBV is the familiar abbreviation for human hepatitis B virus, although with HAV and HCV, a major cause of liver damage and morbidity. LHBsAg, MHBsAg and SHBsAg are the abbreviations for the large, middle-sized and small versions of the viral surface antigen, the proteins involved in the initial phase of virus surface attachment and infection.

Peer-review

The paper is well written.

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