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Transcriptional activity comparison of different sites in recombinant Marek's disease virus for the expression of the H9N2 avian influenza virus hemagglutinin gene

Fushou Zhang[a], Wenqing Chen[a], Chengtai Ma[a], Zhenjie Zhang[b,c], Peng Zhao[a], Yan Du[a], Yaoyao Zhang[a], Luntao Duan[a], Jian Fang[a], Sifei Li[a], Hongqin Su[a], He Cui[a], Zhizhong Cui[a,*]

[a] College of Veterinary Medicine, Shandong Agricultural University, Tai'an 271018, China
[b] Collaborative Innovation Center for the Origin and Control of Emerging Infectious Diseases,Taian 271016, Shandong, China
[c] Institute of Pathogen Biology, Taishan Medical College,Taian 271016, Shandong, China

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A B S T R A C T

Over the last two decades, much attention has been paid to MDV-vectored recombinant vaccines. Many factors have influenced their protective efficacy, and insertion site has been among the main influential factors for the expression of foreign genes in recombinant Marek's disease virus (rMDV). To compare the transcriptional activity of different sites of rMDV, an H9N2 avian influenza virus hemagglutinin gene (AIV-H9N2-HA) expression cassette that used the bi-directional promoter of serotype 1 MDV (MDV1) in the 1.8 kb RNA transcript direction (p1.8 kb) as a promoter was inserted into 4 different regions of MDV using the bacterial artificial chromosome (BAC) vector and FLP/FRT recombination technique. The insertion regions included 3 of its own sites (US2, US10 and one of Meq genes) in the MDV genome and a foreign site (gpt gene) in the BAC vector. Quantitative PCR and enzyme-linked immunosorbent assay (ELISA) were used to analyze and compare the H9N2-HA expression levels of these different rMDVs both at the mRNA level and at the protein level. The results indicated that among the four tested insertion regions, the HA expression cassette in the US2 region demonstrated the highest activity, followed by that in the Meq region, which was almost equal to that of US10. Further, the expression cassette had the lowest activity in the foreign region gpt gene. The above data could be useful for choosing proper recombinant insertion regions in the construction of rMDV to express different foreign genes, and it is a prerequisite for developing effective MDV-vectored recombinant vaccines.

1. Introduction

Marek's disease (MD), which is caused by Marek's disease virus (MDV) of serotype 1, exists in almost all countries as a common lymphoproliferative and immunosuppressive disease in chickens (Churchill and Biggs, 1967; Nazerian et al., 1968). MDV, a highly contagious disease virus, is divided into 3 serotypes (serotypes 1, 2 and 3), according to the distinction of antibodies to the epitopes. Thus, the 3 serotypes can be identified by the specific monoclonal antibody. MD can be controlled with commercial live MDV vaccines, including herpesvirus of turkey (HVT) of serotype 3 (Okazaki et al., 1970), SB-1 strain of serotype 2 (Schat and Calnek, 1978) and CVI988/Rispens of serotype 1 (Rispens et al., 1972). All of the MDV vaccines of different serotypes have been considered among the vectors with the greatest potential for polyvalent vaccines, based on the recent years' research (Heckert et al., 1996; Reddy et al., 1996; Hirai and Sakaguchi, 2001), and recombinant HVT expressing the infectious bursal disease virus (IBDV) VP2 gene has been licensed and made available commercially (Darteil et al., 1995; Bublot et al., 2007).

The genomes of MDVs of different serotypes are very large, and there are many non-essential regions for viral growth in culture, which can be chosen to insert and express foreign antigens in their enormous genomes (Cantello et al., 1991; Parcells et al., 1994; Sakaguchi et al., 1994; Tsukamoto et al., 2002). Therefore, the choice of insertion regions is among the major factors that influence protective efficacy. US2 and US10 have usually been chosen as the insertion regions for most MDV recombinants. It was reported that expression of the HA of H5N1-avian influenza virus (AIV) at the US2 insertion site of HVT induced better protection than that at the US10 insertion site, and the plaque formation and growth kinetics were also different in chicken embryo fibroblasts (CEFs)

* Corresponding author. Tel.: +86 18605381906.
E-mail address: zzcui@sdau.edu.cn (Z. Cui).

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infected with the two recombinant viruses (Gao et al., 2011). Either a Meq-deleted attenuated MDV1 strain GX0101ΔMeq, which was constructed by Li et al. (2011b), or Meq-deleted Md5, constructed by Lee et al. (2013), could provide protection for chickens against MD. Both of the Meq genes might be good region candidates to insert foreign protective genes, although there have been no relative reports about the protective efficacy of recombinant MDV expressing foreign protective genes in these regions. In this study, a foreign insertion site was also chosen as the research object. The gpt gene, which is part of the BAC vector, was used as a selective marker gene for the construction of a BAC clone of MDV1 strain GX0101 (Sun et al., 2009). Therefore, it is absolutely non-essential for viral growth both in vitro and in vivo theoretically.

AIV, which can lead to avian influenza, is divided into many serotypes on the basis of the envelope glycoprotein hemagglutinin (HA, 16 subtypes) and neuraminidase (NA, 9 subtypes). The HA and NA decide the antigenic variation, host scope (human or animal), pathogenicity (highly pathogenic avian influenza virus [HPAIV] or highly pathogenic avian influenza virus [LPAIV]), immunity and epidemiology. Using MDV of different serotypes as vectors, over the last several years, many recombinants expressing one HA gene or two genes (HA and NA) of different serotypes of AIV have been constructed, and some of them could protect chickens from AIV challenge completely, as well as MDV (Li et al., 2011a).

In this study, a Meq-deleted GX0101 of a BAC-cloned strain of MDV1 (Li et al., 2011b) was used as a vector to express the HA gene of H9N2 of AIV under the control of MDV1's own bi-directional promoter in the 1.8 kb RNA transcript direction (p1.8 kb), which was inserted at the different sites. The transcription and expression levels of the HA gene were compared in cell cultures. In addition to the US10, US2 and Meq regions, the gpt site, as a foreign region belonging to the BAC system, was also used and was compared at the transcriptional level in these recombinant MDVs.

2. Materials and methods

2.1. Viruses and cells

The parental virus was GX0101ΔMeq, which both of the Meq genes were deleted, and it was constructed based on the BAC system. EL250 was used as the host *E. coli* for the whole genomes of recombinants. The H9N2 LG1 strain was maintained in Poultry Phymatosis laboratory of Shandong Agricultural University. Chicken embryo fibroblast cells (CEFs) that were prepared from 10-day-old specific-pathogen-free (SPF) embryonated chicken eggs were used to transfect eukaryotic expression plasmid and to cultivate recombinants. The 1.8 kb mRNA transcript direction of bi-directional promoter (p1.8 kb) was used to transcript the H9N2-HA gene into these recombinants, and they were cloned from the parental virus GX0101ΔMeq.

2.2. Primers

As listed in Table 1, these primers were used to construct and detect the recombinant MDVs. The reverse primers R (H-Meq), R (H-GPT), R (H-US2) and R (H-US10) were used with the same forward primer F9 to replicate the downstream chimera fragments of the recombinants of different integrated regions, respectively. The underlined cases are the restriction sites that were used for cloning. The lower-case letters in P1 (Meq), P2 (Meq), P3 (GPT), P4 (GPT), P5 (US2), P6 (US2), P7 (US10) and P8 (US10) indicate the homology arms that used to recombine with GX0101ΔMeq, and the capital letters indicate the PCR primers that were used to replicate the HA expression cassettes.

2.3. Construction of H9N2-HA eukaryotic expression plasmid and detection with IFA

Using the pair of F(HA) primers containing a Nhe I restriction site (underlined) and R(HA), which contained a BamH I restriction site (underlined) as indicated in Table 1, the complete coding region of the H9N2-HA gene was amplified by RT-PCR from a AIV-H9N2 viral RNA genome and then was cloned into the eukaryotic expression vector pcDNA3.1(−). The fragment of H9N2-HA ORF plus Poly A was amplified using F(HA-PyA) and R(HA-PyA) from the constructed eukaryotic expression plasmid, and the p1.8 kb was amplified by PCR using primers F (p1.8 kb) and R (p1.8 kb) from GX0101Δmeq. Both of them were cloned into the PMD18-T vector (T-p1.8 kb and T-HAORF) and were sequenced. Then, the H9N2-HAORF was cloned into the T-p1.8 kb using the Not I and Nhe I restriction sites

Table 1
Primers used for amplification of different gene sequences or fragments for construction or detection of recombination MDVs in this study.

Primers	Sequences 5' to 3'	Target genes or fragments
F (p1.8 kb)	TCTAGATCAGGGCCCTCGAGGCCACAAGAAATTAC	Promoter of p1.8 kb
R (p1.8 kb)	GCGGCCGCGATGCTAGCGAGCATCGCGAAGAGAGAAG	
F(HA)	GCTAGCATGGAAACAATATCACCAATAG	The H9N2-HA ORF
R(HA)	GGATCCTTATATACAAACGTTGCATCTGC	
F(HA-PyA)	GCTAGCATGGAAACAATATCACCAATAG	The H9N2-HA ORF plus Poly A
R(HA-PyA)	GCGGCCGCTTATATACAAACGTTGCATCTGC	
F(kana)	TCTAGACGTGTAGGCTGGAGCTGCTTC	The Kana expression cassette
R(kana)	GGGCCCCATTCCGGGGATCCGTCGAC	
P1(Meq)	tgcaggtgtataccagggagaaggcgggcacggtacaggtgtaaagagCGTGTAGGCTGGAGCTGCTTC	The linear expression cassettes flank
P2(Meq)	agaaacatggggcatagacgatgtgctgctgagagtcacaatgcggatcCCATAGAGCCCACCGCATCC	with 50bp homology arms of Meq
P3(GPT)	ggatctcccgcccagcgtcttgtcattggcgaactcgaacacgcagatgCGTGTAGGCTGGAGCTGCTTC	The linear expression cassettes flank
P4(GPT)	ttagcgaccggagattggcgggacgaatacgacgcccatatcccacggctCCATAGAGCCCACCGCAT	with 50bp homology arms of GPT
P5(US2)	cgattatgggcacacccacatcatcctgtatttgttccatacattgctttCGTGTAGGCTGGAGCTGCTT	The linear expression cassettes flank
P6(US2)	ctagatgaatgcgatcgattgccaggaagatctagagatgctgcatctacCCATAGAGCCCACCGCATCC	with 50bp homology arms of US2
P7(US10)	cccgacgagaaaaattgtaggccgggctttacggtgtattgaacgtgctcCGTGTAGGCTGGAGCTGCTTC	The linear expression cassettes flank
P8(US10)	ttataagtaggattccccgtctcctgttggcgattcccgaagatttgtcaCCATAGAGCCCACCGCATCC	with 50bp homology arms of US10
F9	GAAGGGGTCAAGCTGGAATCTGA	The downstream chimera fragments of
R(H-Meq)	GGAACTCCTGGAGCCAACAAAT	recombinants of Meq region
R(H-GPT)	GCGGATGAATGGCAGAAATTCGA	GPT integrated region
R(H-US2)	ATGGGTGTGTCCATGATAACTAT	US2 integrated region
RQHA-F	ATCTGGGACATCCTCTCATTCTAA	Target gene (H9N2-HA) of real time
RQHA-R	ACATTCCCGGGGTAACACAT	relative quantification
RQpp38-F	GACGCGTTCGCACTGCTCATTTG	Endogenous gene (GX0101-pp38) of
RQpp38-R	CGTTGCCGTTCGATCCAGGTCTC	real time relative quantification