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Research article

CLONING OF CHICKEN ANEMIA VIRUS VP3 GENE IN pVAXI VECTOR

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ABSTRACT: Chicken anemia virus (CAV) is an important viral pathogen that causes anemia and severe immunodeficiency syndrome in chickens. The VP3 protein of CAV also named as "apoptin" possesses the ability to induce apoptosis in transformed cells. In this study the VP3 gene obtained from pTargeT.cav.vp3 was cloned in pVAXI vector. RE analysis with *PstI*, colony PCR and sequencing results suggest that the gene was present in right orientation in the pVAXI plasmid vector. Since pVAXI is approved by United States Food and Drug Administration for use in human, it can be used in trial for its antitumor activity.

KEYWORDS: VP3, pVAXI vector, pTargeT vector, Chicken anemia Virus, apoptin, apoptosis, cloning.

INTRODUCTION

Chicken anemia Virus (CAV) is causative agent of chicken infectious Anemia. CAV was first isolated in 1979 in Japan and is the major agent responsible for a disease causing severe anemia and immunosupression (Yuasa *et al*, 1979). CAV is the member of the genus Gyrovirus of family Circoviridae and the genome consist of circular single stranded 2.3kb DNA molecule (Meehan *et al*, 1992). CAV possesses three types of protein VP1, VP2 and VP3. VP1 (45-52 kDa) is the major structural capsid protein, encapsidates a negative strand genome of about 2300 bases (Allan *et al*, 2003; Todd *et al*, 2007). VP2 (24kDa) is a dual specificity protein phosphatase and has

been shown to interact with VP1 (Goldbach *et al*, 2007). The third viral protein VP3, also named "apoptin" is a strong inducer of apoptosis in chicken thymocytes and chicken lymphoblastoid cell line (Jeurissen *et al*, 1992). The tumor-specific apoptotic characteristic of the protein potentially may allow the development of a drug that has applications in tumor therapy (Lee *et al*, 2012). Hence cloning of VP3 in pVAXI vector was undertaken.

MATERIALS AND METHODS

Vector

The vector pVAXI and pTargeT.cav.vp3 recombinant plasmid were available in Biotechnology Laboratory, IBIT, Bareilly. The pVAXI was used to subclone the VP3 gene of CAV and construct recombinant mammalian expression vector for the study of apoptosis.

Gene

The VP3 gene of CAV used for this study was released from recombinant plasmid pTargeT.cav.vp3.

Bacterial Strain

E.coli strain DH5α (Promega, Madison) was used for transformation with recombinant plasmid.

Primers

The primers used for this study were VP3F 5' ATG AAC GCT CTC CAA GAA G3' (19 mer) and VP3R 5' CTT ACA GTC TTA TAC ACC TT 3' (20 mer).

Virus

Chicken anemia virus used for study was available in IBITCC of IBIT Bareilly.

Plasmid DNA isolation

E.coli DH5α containing pTargeT.cav.vp3 recombinant plasmid was grown in LB Broth containing ampicillin 100 µg/ml and plasmid DNA was isolated using TELT method (Ausubel *et al*, 1990). Similarly *E. coli* DH5α containing pVAXI vector was grown in LB broth containing kanamycin 25µg/ml and plasmid DNA was isolated using TELT method.

Release of VP3 gene and preparation of pVAXI vector

The pTargeT.cav.vp3 recombinant plasmid was digested with *NheI* and *KpnI* enzyme to release the VP3 gene. Similarly pVAXI vector was also digested with *NheI* and *KpnI* enzyme at 37^0C overnight. 10ul of both digested DNA were run on 1.5% agarose gel using 1X TAE running buffer system containing ethidium bromide and

visualized under UV transilluminator. The pVAX1 DNA band was cut and kept in a microfuge tube. Similarly VP3 band was cut and kept in a microfuge tube. The pVAX1vector and VP3 were extracted from gel using phenol-chloroform .The pVAX1 was dephosphorylated using calf intestinal alkaline phosphatase using method of Sambrook and Russel, 2001.

Ligation of VP3 and pVAX1

One µl pVAXI Plasmid vector, 2µl VP3 gene insert, 1µl T4 DNA ligase, 1µl ligase buffer and 5µl nuclease free water were added in a microfuge tube making a total 10µl reaction mixture. The reaction mixture was mixed by vortexing and spun to settle the ingredients in bottom and incubated overnight at 14^0C.

Competent cell preparation and transformation

The *E.coli* DH5α was streaked on LB agar plate and incubated overnight at 37^0C. Single colony was inoculated in 5 ml LB broth and incubated overnight at 37^0C. Now equal volume of chilled 2XTSS was added to cell Suspension (Chung *et al,* 1989). 5µl of ligated product was mixed with 200µl cell suspension and incubated on ice for 30 min and after incubation heat shock was given at 42^0C for 50 Sec. Immediately return it on ice for 2 min. Then spread the transformants on LB agar plate containing kanamycin 25µg/ml with spreader and incubated at 37^0C overnight. About 5 colonies were picked up and inoculated in LB broth and plasmid DNA were is isolated.

RE analysis

The recombinant plasmid DNA sequence was analysed by DNAstar MapDaw software and *PstI* enzyme was found to be appropriate to detect the right orientation of the gene which is expected to produce fragments of size 3.026 kb and 0.345 kb.

PCR

The presence of VP3 gene insert in right orientation in pVAXI recombinant plasmid was confirmed by PCR using T7 Primer as forward primer and gene specific reverse primer.

Sequencing

The recombinant plasmid was further confirmed for gene in right orientation by sequencing with T7 sequencing primer.

RESULTS AND DISCUSSION

To clone the CAV VP3 gene in pVAXI vector, the VP3 gene was successfully released from recombinant plasmid pTargeT.cav.vp3 by digesting with *NheI* + *KpnI* enzyme (Fig. 1) and

purified from the agarose gel. pVAXI was digested with *NheI +s KpnI* and dephosphrylated. The vp3 gene insert was ligated to pVAXI expression vector using T4DNA ligase and the ligation mixture was transformed in *E.coli* DH*5α* and plated on LB agar containing kanamycin. Large numbers of colonies were observed but one colony was used for plasmid DNA isolation. The recombinant plasmid digested with *PstI* produced two fragments of size 3.026 kb and 0.345 kb (Fig. 2).These sizes were expected if the insert is in right orientation as analysed by DNAstar Mapdraw software and thus it was confirmed that the VP3 gene was in right orientation. Colony PCR was done using T7 primer as forward primer and VP3 gene specific reverse primer and a product of 372 bp was obtained which confirmed the presence of VP3 gene in correct orientation (Fig. 3). Sequencing with T7 primes further confirmed the presence of gene in right orientation.

VP3 protein of CAV induces apoptosis of tumor cells and it is named as "apoptin". Many studies have been done that provided insight into cloning of CAV. (Lee *et al*, 2007) showed the effect of VP3 gene of chicken anemia virus on canine mammary tumor cells. They observed that the VP3 of CAV induced apoptosis in malignant CMT cells but not in non-neoplastic canine MGE cells. (Natesan *et al*, 2006) reported the antineoplastic effect of chicken anemia virus VP3 protein (apoptin) in Rous sarcoma virus induced tumors in chickens. The result of this study showed that apoptin had an antineoplastic effect *in vivo* and *in vitro* in RSV induced tumors. The antineoplastic effect is due to apoptin induced apoptosis. (Lee *et al*, 2012) also reported the efficient production of engineered apoptin in recombinant *E. coli* for tumor therapeutic application. They observed that the *E. coli*-expressed GST-TAT- apoptin showed apoptotic activity and was able to induce human premyelocytic leukemia HL -60 cells to enter apoptosis. (Wu *et al*, 2012) reported that apoptin enhanced the oncolytic properties of New Castle disease virus. They observed that compared to parental strain FMW, rFMW/AP was more potent in killing A459 and SMMC7721 tumor growth in mice bearing A549-induced tumors. Furthermore, FMW/AP did non display apparent toxic effects in either normal cells or control mice. sThe result suggested that the recombinant NDV expressing apoptin is a promising novel antitumor agent.

Our findings have thus shown that the recombinant plasmid Pvax1.cav.vp3 can be used to evaluate it for its antitumor activity.

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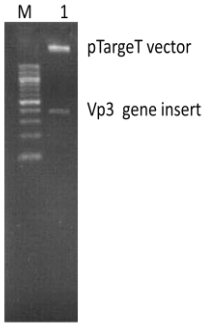

Fig.1. pTargeT.ca-vp3 digested with NheI and KpnI to release the vp3 gene insert.
Lane M: 100bp DNA ladder
1: vp3 gene insert and pTargeT released

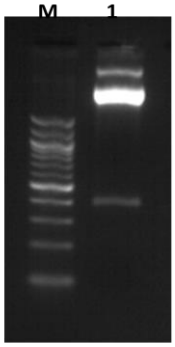

Fig.2. D**igestion of pVAXI.cav-vP3 with PstI releasing fragments 3.026 kb and 0.345 kb confirming the right orientation of the gene.**
Lane M: 100 bp ladder, 1: Release of VP3

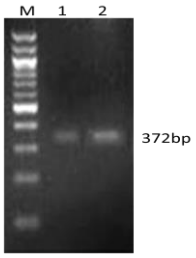

Fig.3. PCR amplification of vp3 gene using plasmid DNA of transoformed colony using T7 forward primer and vp3 gene specific reverse primer.
Lane M: M-100bp DNA ladder;
1 &2- 372 bp VP3 gene amplified

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