IJPSR (2014), Vol. 5, Issue 6 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 January, 2014; received in revised form, 13 April, 2014; accepted, 12 May, 2014; published 01 June, 2014

EXPRESSION OF CHICKEN ANEMIA VIRUS VP3 GENE IN HeLa CELLs

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Keywords:

VP3 gene, pVAX1 vector, expression ,HeLa cells, Apoptin, Chicken anemia virus

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ABSTRACT: Chicken anemia virus (CAV) encodes the protein VP3 also named "apoptin" that specifically induces apoptosis in human tumor cells. In this study, the VP3 gene cloned in p VAX1 plasmid vector was transfected in HeLa cells. The expression of CAV VP3 gene was detected using immunoperoxidase test and indirect fluorescent antibody technique. High level of gene expression was observed in HeLa cell culture. Successful expression of recombinant plasmid in mammalian cell lines shows that the plasmid could be further evaluated in field trials and hence useful tool for study of antitumor activity.

INTRODUCTION: Chicken anemia virus possesses three types of protein i.e. chicken anemia virus VP1, VP2 and VP3. The chicken anemia virus derived protein Apoptin/vp3 (CAV-APOPTIN) has the important ability to induce tumor selective apoptosis in a variety of human cancer cells [1]. Apoptin can induce apoptosis in cell lines derived from great variety of human tumours e.g. hepatoma, lymphoma, carcinoma, leukemia, melanoma, breast and lung tumour and colon carcinoma [2].

Expression of chicken anemia virus VP-2 gene was demonstrated in *E. coli*. In this study CAV-VP2 gene was inserted in Pgex-5x-3 vector then expressed by fusion protein in *E. coli*. The expression was detected on CAV infected MSBI cells by indirect immunoflourocent assay (IFA). The result showed that the expressed protein retained some antigenicities of nature CAV-VP2 and could be used for further study on biological activities of CAV VP2 [3].

The expression of chicken anemia virus VP3 gene and induction of apoptosis in transformed and tumor cells was studied. The differential activities of VP3 were investigated by use of immuno-florescence technique. The VP3 protein was expressed and detected only in the cytoplasm of normal cells. In contrast, the expression of VP3 protein was localized particularly in the nucleus of transformed cell lines (Vero and rat embryo fibro blast cells) and human derived breast cancer cells (MCF-7 and MDAMB 231). This differential cellular localization of VP3 protein in normal verses tumorigenic and transformed cells was the reason of VP3 specifically inducing apoptosis in transformed and cancerous cells but not in normal cells. The conclusion of this study was that the VP3 protein expression alone was able to induce apoptosis in transformed cells and in human derived cancer cells but had no effect on normal cells [4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(6).2525-28

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(6).2525-28

Hence, in this study, the expression of CAV-VP3 gene in HeLa cells using IPT and IFAT was undertaken.

MATERIALS AND METHODS:

Gene: The recombinant plasmid pVAX1.cav.vp3 [5] was used in the present study.

Cells: The HeLa cells used in this study were grown in DMEM (Gibco, NY) with 50µg/ml gentamycin (Amresco, USA) and 10% fetal calf serum (Hyclone, USA) in 25 square cm plastic flasks.

Hyperimmune serum: Hyperimmune serum against VP3 gene was raised in mice using recombinant plasmid pVAX1.cav.vp3.

Transfection: The calcium phosphate DNA-coprecipitate was prepared by combining CaCl2 with plasmid DNA in sterile microfuge tube and added 80µl DW in it and kept for 1 minute. Immediately transferred 20µl calcium phosphate-DNA suspension in each well of 96 well microtiter plate.

Then 100µl of HeLa cells suspension was added into each well. Keep control well without transfection. The plate was incubated at 37°c for 72 hours at 5% Co2 tension and humidity [6]. After incubation the cells were assayed for expression of the transformed gene using IFAT and IPT technique.

Indirect Immunoflourescent Assay: After 72 hrs of transfection, the medium in 96 well plates was poured off and washed with PBS and fixed with 4% paraformaldehyde. The mock transfected HeLa cells were also fixed as control. To the fixed monolayer, 1:50 diluted mouse anti VP3-CAV hyperimmune serum was added and incubated at 37°C for 1 hr.

After incubation the wells were washed with PBS again incubated with goat-anti mouse FITC conjugated secondary antibody and incubated further for 1hr at 37°C. Thereafter, wash the cell monolayer with PBS, mounted in 5% glycerol in PBS and examined under fluorescent microscope (Nikon, Japan) and photographed.

Immunoperoxidase test: The initial method upto transfection was as described under FAT method. After 72 hrs of transfection medium was removed, wells washed with PBS and then fixed with chilled acetone at 4°C for 1 min and air dried. Added few drops of anti-cav-vp3 hyperimmune serum and incubated at 37°C for 1 hrs in humid chamber. Then washed with PBS and put few drops of goat anti-mouse-HRP and incubated at 37°c for 1 hr then rinsed the wells with PBS and air dried. Put 2-3 drops of Nadi reagent and allowed to react for 2-5 mins and then rinsed with PBS .Treated for 1 min with each 70%, 90%, and 100% alcohol followed by quick wash with xylene and mounted with DPX mountant. Observed under microscope and photographed.

RESULTS: Successful expression of chicken anemia virus VP3 gene in HeLa cells was shown. The expression of VP3 gene was done by using pVAX1 .cav.vp3 recombinant plasmid by transfecting HeLa cells.

The expression of VP3 gene recombinant plasmid was confirmed by Indirect immunoflourescent assay and immunoperoxidase test. The immunoflourescent analysis of CAV VP3 gene transfected HeLa cells revealed intracytoplasmic flourescence in transfected cells (**fig. 1**) while there was no flouresence in control (**fig. 2 &3**), which confirmed the expression of CAV. VP3 gene. Similarly recombinant plasmid transfected HeLa cells showed intense positive color reaction on IPT (**fig. 4**), while control did not show any color development (**fig. 5 & 6**). About 90% of the cell monolayer showed positive color development indicating high level of gene expression.

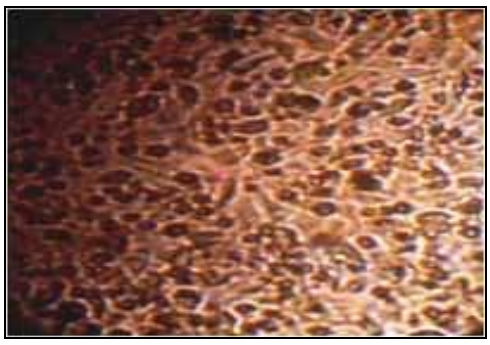

FIG. 1: EXPRESSION OF CAV-VP3 IN HeLa CELLS ANALYSED BY IMMUNOPEROXIDASE TEST SHOWING POSITIVE COLOR DEVELOPMENT

FIG. 2: VECTOR ALONE TRANSFECTED HeLa CELLS SHOWING NO EXPRESSION

Similarly recombinant plasmid transfected HeLa cells showed intense positive color reaction on IPT (**fig. 3**), while control did not show any color development (**fig. 4**). About 90% of the cell monolayer showed positive color development indicating high level of gene expression.

FIG. 3: MOCK- TRANSFECTED HeLa CELLS SHOWING NO EXPRESSION

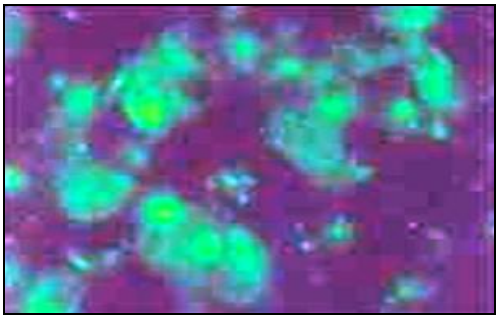

FIG. 4: EXPRESSION OF CAV-VP3 IN HeLa CELLS ANALYZED BY IMMUNOFLUORESCENCE TEST SHOWING POSITIVE REACTION

FIG. 5: VECTOR ALONE TRANSFECTED HELA CELLS SHOWING NO EXPRESSION

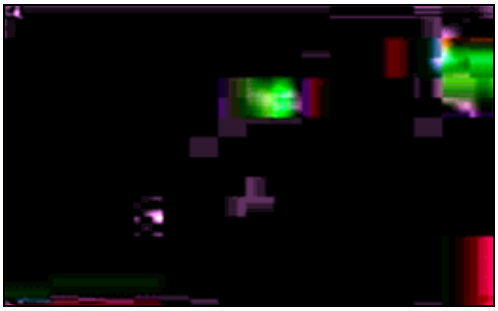

FIG. 6: MOCK TRANSFECTED HeLa CELLS SHOWING NO EXPRESSION

DISCUSSION: Many studies have been done that provided insight into expression of CAV VP gene. The expression and characterization of highly antigenic domains of chicken anemia virus viral VP2 and VP3 subunit proteins in a recombinant E.coli for sero-diagnostic applications was studied. The results indicated that VP2 and VP3 show good immunoreactivity with CAV-positive chicken sera, whereas VPI was found to be show less immunoreactivity than VP1 and VP2 [7].

The expression of chicken anemia virus was demonstated by other workers also [8, 3].

It has been demonstrated that the VP3 protein expression alone was able to induce apoptosis in transformed cells and in human derived cancer cells but had no effect on normal cells [4].

The production of chicken anemia virus (CAV) VP1 and VP2 protein expressed by recombinant Escherichia coli were observed [9].

Thus our observations are in full agreement with the results obtained by other workers.

CONCLUSION: Successful expression of VP3 gene in HeLa cells was done and it is concluded that the gene shows high level of expression. It shows that the gene can be used for further studies in field of antitumor activity.

ACKNOWLEDGEMENT: The author are thankful to the vice chancellor, Mewar University, Rajasthan and Institute of Biotechnology & IT, 197, Mudiya Ahmadnagar, Bareilly, U.P. India for providing necessary research facilities for carrying out this work.

REFERENCES:

1. Bullen kamp J,Cole D, Malik F, Kulasekararaj A ,Odell EW , Farzaneh F, Gakeh J , Tavassoli. M: Cell death and disease. 2012; 296: doi:10.1038/cddis.2012.34.
2. Noteborn M H M, De Boer G F, Van Roozelaar D J, Kaman C, Kranenburg O, Jeurissen S H M, Hoeben R C, Zantema A, Koch G, Van Ormondt H , Van Der Eb A J. Characterization of cloned chicken anemia virus DNA that contains all element for the infectious replication cycle. J.Virol. 1991; 65:3131-3139.
3. Liu L Y, Jian Q A,Jie J W, Quiang Y J,Rong J I, Zhong C H, You D Y. Expression of chicken anemia virus (CAV) VP2 gene in E.coli and its biological activities.(2003-01). (The animal science and veterinary college, Yangzhou University, Yangzhou 225009, China).
4. Ghrici, Mohamed .The expression of chicken anemia virus VP3 gene and induction of apoptosis in Transformed and Tumor cells. Master Thesis. 2001; University Pertanian Malaysia.
5. Maurya R, Rai A. Cloning of chicken anemia virus VP3 gene in pVAX1 vector. Biotechnology International. 2013; 6 : www.bti.org.in
6. Rai A, Tiwari A K, Gupta P K, Madhan Mohan C, Raut A A, Kumar S, Rai N, Gangwar S. Laboratory Manual of Biotechnology.2010 ;3. www.bts.org.in.
7. Lai G H, Lin M K, Lien Y Y, Fu J H, Chen H J, Huang C H, Tzen J T, Lee M S. Expression and characterization of highly antigenic domain of chicken anemia virus viral VP2 and VP3 subunit proteins in a recombinant E.coli for sero-diagnostic application..BMC Vet Res. 2013; doi: 10.1186/1746-6148-9-161.
8. Lacorte C , Lohuis H, Goldbach R , Prins M.Assessing the expression of chicken anemia virus .Virus Research. 2007; 12: 80-86.
9. Lee M S,Lein Y Y, Feng S H,Huang R L,Tsai M C,Chang W T,Chen H J., Production of chicken anemia virus (CAV)VP1 and VP2 protein expressed by recombinant Escherichia coli. 2008 ;doi:10,1016/J.Proc bio.2008-11.016.Lee M S, Lein YY, Feng SH, Huang RL, Tsai MC, Chang WT, Chen HJ. Production of chicken anemia virus VP1 and VP2 protein expressed by recombinant *Escherichia coli*. 2008;.doi:10.1016/Proc bio 2008-11.016.

How to cite this article:
Maurya R and Rai A: Expression of Chicken Anemia Virus VP3 gene in HeLa CELLs. Int J Pharm Sci Res 2014; 5(6): 2535-38.doi: 10.13040/IJPSR.0975-8232.5(6).2525-28