Porphyromonas gingivalis FimA Type II - PVXCP Fusion DNA Vaccine Expression in Mammalian Cells

Jantipa Jobsri* Wanvisa Piluek Ponjarus Pesoongnern** Kusuma Jamdee****

Abstract

Objective: It has been shown that *Porphyromonas gingivalis (P. gingivalis)* FimA is able to induce antibody production and protection against bone loss in animal model. *P. gingivalis* FimA is therefore the candidate antigen in periodontitis vaccine production. In this study, DNA vaccine was produced by fusion sequences of FimA type II, the most prevalent type associated with periodontitis, and potato virus X coat protein (PVXCP), the immune-enhancing molecule. The ability of this DNA vaccine to be expressed in mammalian cells was investigated.

Methods: The DNA vaccine was constructed in a fusion form of FimA and PVXCP (FimA -PVXCP) DNA vaccine, in which the mammalian expression plasmid pcDNA3 was used as a backbone plasmid. FimA type II gene was amplified from genomic DNA of P.gingivalis FimA type II by PCR. The FimA PCR product was inserted into predigested pcDNA3 containing PVXCP sequence; and consequently FimA was fused to the N-terminal side of PVXCP. The resulting plasmid was transfected into human embryonic kidney (HEK293) cells. FimA-PVXCP RNA expression in transfected cells was detected by RT-PCR. The fusion protein inside the cells and the protein secreted into medium were analyzed by Western Blot analysis using anti-PVXCP antibody.

Results: RT-PCR of RNA extracted from pcDNA3. FimA-PVXCP transfected HEK293 cells showed the expected band size of 1.9 kb of FimA-PVXCP sequence. FimA-PVXCP protein was detected by Western blot analysis both in cell lysate of the transfected cells and in the medium. However, the secreted protein appeared to be larger than the protein remained inside the cells.

Conclusion: FimA-PVXCP DNA vaccine was able to be expressed in HEK293 cells as confirmed by RT-PCR and Western blot analysis.

Keywords : *P.gingivalis* vaccine, Periodontitis vaccine, DNA vaccine, Protein expression in mammalian cells

**Lecturer, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand.*

***Research Assistant, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand.*

Introduction

Porphyromonas gingivalis (P. gingivalis), a non-motile asaccharolytic gram-negative rod anaerobic bacterium residing in human oral cavity, is a major pathogen causing periodontitis [1, 2] which is a common disease but resulting in severe damaging conditions. Periodontitis is a major cause of tooth supporting tissue destruction and adult tooth loss. Five to eighty percent of population world-wide was suffered from periodontitis; the percentage varies among nationalities and age groups with the prevalence increased over the age [3]. The study in 998 Thai monks found that approximately 12.7 % of the studied population had periodontitis with 41.4% of priests age of 55 - 64 years old suffered from the disease [4].

Apart of its role in periodontal disease, *P. gingivalis* is also associated with other systemic diseases and conditions such as aspiration pneumonia, rheumatoid arthritis and cardiovascular disease [5-10]. It was found that subjects with aspiration pneumonia, an important cause of morbidity and mortality in persons age 60 and older, had higher amount of *P. gingivalis* in dental plaque [11]. P. gingivalis could also be detected in atheromatous plaque specimens collected during surgery of cardiovascular disease patients [9] and in synovial fluid collected from rheumatoid arthritis patients [10].

As *P. gingivalis* is a pathogen causing periodontitis and other systemic diseases, researchers have studied on feasibility to produce vaccines against it. *P. gingivalis* major fimbriae or pilli is one of the candidate antigens. Major fimbriae subunit, FimA, was administered into mice and able to induce fimbriae-specific salivary IgA and IgG and serum IgG in the immunized mice [12, 13]. In addition, the immunized mice were protected from alveolar bone loss after challenging with *P. gingivalis* [13]. These evidences show the possibility of using *P. gingivalis* vaccine as a periodontitis vaccine.

DNA vaccines have long been proved to be able to induce protective and specific immunity against pathogens or cancers. In some cases, the candidate antigens, such as tumour antigens, are weak immunogens. One of the strategies to improve immunity against weak antigens is to fuse the antigens to immune-enhancing molecules. Potato virus X coat protein (PVXCP) has been proven to be a highly immune-enhancing molecule in tumour vaccines. PVXCP provided help to enhance specific antibody production and provided protection against tumour. Protection effect of the vaccine appeared to involve CD4 T-cell response [14].

As it has been shown that *P. gingivalis* FimA induced antibody production and protection against bone loss, *P. gingivalis* FimA is therefore the candidate antigen used in this project. The strategy for the vaccine production is to fuse the sequence of FimA genotype II, the most prevalent type associated with periodontitis, to the sequence of PVXCP. The fusion of PVXCP to FimA is aimed to induce strong immune response.

In this study, the DNA vaccine was constructed and transfected into mammalian cells to investigate the ability of the vaccine to mediate antigen production and secretion.

Materials and Methods

DNA vaccine construction

The DNA vaccine was constructed in a fusion form of FimA and PVXCP (FimA-PVXCP), in which the mammalian expression plasmid pcDNA3 was used as a backbone. FimA type II gene was amplified from genomic DNA of *P.gingivalis* FimA typeII isolated in our laboratory. Genomic DNA was purified using QIAamp® DNA Mini kit (QIAGEN, Hilden, Germany). For FimA type II PCR (Thermo Scientific Phusion Hot Stat II High-Fidelity DNA Polymerase, Thermo Scientific, Waltham, MA, USA), PCR primers contain ApaLI site at the 5' end of the forward primer (FimIIApaLIFWD primer; 5' TCATGTGCACTC-GATGAAAAAAACAAAGTTTTTCTTGTTGGGACTT 3') and BspEI site at the 5'end of the reverse primer (FimIIBspEIREV primer; 5' TATTCCGGACCAAG-TAGCATTCTGACCAACGAG 3'). The PCR product was isolated by agarose gel electrophoresis and gel extraction (Thermo Scientific GeneJet Gel Extraction Kit, Thermo Scientific).

FimAtypeII gene PCR product was then ligated to leader sequence amplified from pcDNA3. BCL1scfv-PVXCP (pcDNA3 DNA vaccine containing BCL1 scFv fusion with PVXCP) using forward primer containing HindIII at the 5' end (HindIIIleader F; 5'GTCAAGCTTGTCGACCATGGGCTG 3') and the reverse primer containing ApaLI at the 5' end (ApaLIleaderR primer; 5' GCGAGTGCACACCT-GTGGAGA 3'). The leader-FimA ligated product was amplified using HindIIIleaderF and FimIIBsp EIREV primers. After gel purification, leader-FimA fragment was digested with HindIII and BspEI and subsequently ligated into HindIII/BspEIpre-digested pcDNA3.BCL1scfv-PVXCP. The resulting plasmid was pcDNA3 containing fusion FimA-PVXCP (pcDNA3.FimA-PVXCP) with leader sequence at the 5' end of the fusion sequence.

The ligated plasmids were transformed into JM109 competent cells and the transformed cells were selected using LB plate containing 100 microgram/ml of ampicillin. Colonies grew on the LB plate were screened by colony PCR using DreamTaq™ Green DNA polymerase (Thermo Scientific) with HindIIIleaderF/FimIIBspEIREV primer pair or HindIIIleaderF/PVXREV primer pair (PVXREV: PVXCP reverse primer,5'ACCTCGAGT TATGGTGGGGGTAGTGA3'). The correct fusion sequence was confirmed by DNA sequencing. Then pcDNA3. FimA-PVXCP was purified with Nucleospin® Plasmid (Macherey-Nagel, Duren, Germany) for further transfection experiments.

FimA-PVXCP expressions in mammalian cells

To examine FimA-PVXCP expression in mammalian cells, HEK293 cells was transfected by pcDNA3. FimA-PVXCP with Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA). Plasmid pcDNA3.BCL1scfv-PVXCP was transfected into HEK293 cells as a positive control for protein expression. HEK293 cells with no transfection were used as a negative control. Two days after transfection, cell culture medium was collected and cells were harvested and divided equally for RNA extraction and Western blot analysis. Total RNA from transfected cells was isolated by NucleoSpin® RNA (Macherey-Nagel). First strand cDNA was synthesized using Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) and FimA-PVXCP PCR was performed using DreamTaq™ Green DNA polymerase (Thermo Scientific) with FimIIApaLIFWD and PVXREV primers.

For Western blot analysis, cells were lysed with 85°C pre-heated protein sample loading buffer. Proteins in cell culture medium and cell lysate were separated on 12% SDS-PAGE and were subjected to Western-blot analysis using rabbit anti-PVXCP antiserum followed with HRP-conjugated goat anti-rabbit IgG antibody (Sigma-Aldrich, St. Louis, MO, USA). The protein specific to anti-PVXCP antibody was visualized by Luminata^{TM} Forte Western HRP Substrate (Millipore Corporation, Billerica, MA, USA) and ChemiDoc^{TM} MP imaging system (Bio-Rad, Hercules, CA, USA).

Results

Construction of FimA-PVXCP DNA vaccine

After fragments of Leader-FimA were ligated to PVXCP in pcDNA3 plasmid and the resulting plasmids were transformed into JM109 cells, colonies grew on ampicillin-LB agar was screened by colony PCR. Most of screened colonies, except clone number 3, 9, 12, and 15, showed PCR product with 1.3 kb size of Leader-FimA sequence (Fig. 1). Some clones were randomly selected to further PCR screened for Leader-FimA-PVXCP sequence and all of the selected clones showed correct PCR product size of 2 kb (Fig. 2).

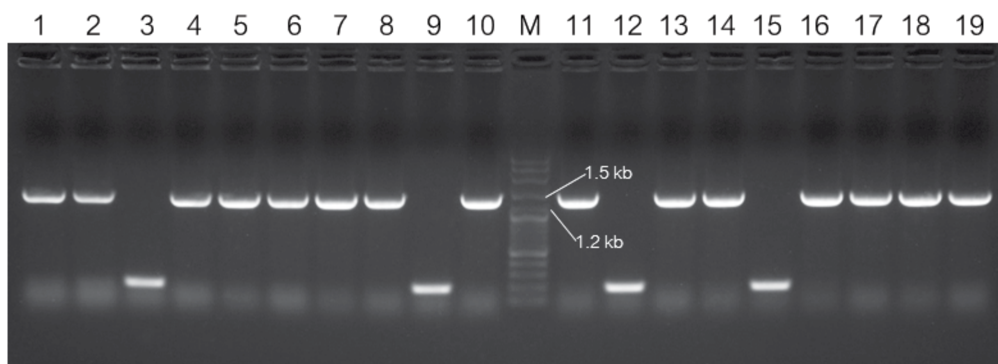

Figure 1. Electrophoresis of Leader-FimA PCR product (1.3 kb). Number 1 – 19 indicate clone number. M: VC 100 bp Plus DNA ladder.

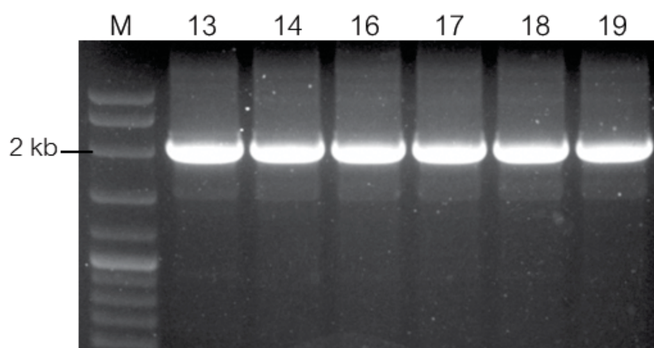

Figure 2. Electrophoresis of Leader-FimAPVXCP PCR product (2 kb). Numbers indicate clone number. M: VC 100 bp Plus DNA ladder.

Clone number 17 was selected for plasmid DNA preparation for further experiment. Correct organization and base composition of leader sequence, FimA, and PVXCP were confirmed by DNA sequencing. In addition, blast analysis revealed that FimA typeII in this DNA vaccine was 100% similar to *P. gingivalis* FimA type II strain TDC117 (NCBI accession number AB195789.1, GI 66796141).

FimA-PVXCP expressions in HEK293 cells

To evaluate the ability of FimA-PVXCP fusion protein to be expressed in mammalian cells, purified pcDNA3.FimA-PVXCP was transfected into HEK293 cells. Transfection with pcDNA3. BCL1scfv.PVXCP was a positive control for protein expression. HEK293 cells with no transfection were used as a negative control. FimA-PVXCP RNA was detected only in cells transfected with pcDNA3. FimA-PVXCP as expected (Fig. 3). RT PCR without reverse transcriptase of HEK293 cells transfected with pcDNA3.Fim-PVXCP showed no PCR product. This data confirmed that the RT-PCR product observed in pcDNA3. FimA-PVXCP transfection was the result from RNA expression not the contaminated plasmid DNA.

From Western blot analysis, FimA-PVXCP protein inside the cells migrated with apparent molecular weight of 80 kDa while the protein secreted into the medium migrated with apparent molecular weight of 120 kDa (Fig. 4). In pcDNA3. BCL1scfv-PVXCP transfection, BCL1scfv-PVXCP inside the cells was 50 kDa as expected and the secreted protein was larger than protein remain in the cells as well. These data confirmed that FimA-PVXCP could be expressed in mammalian cells.

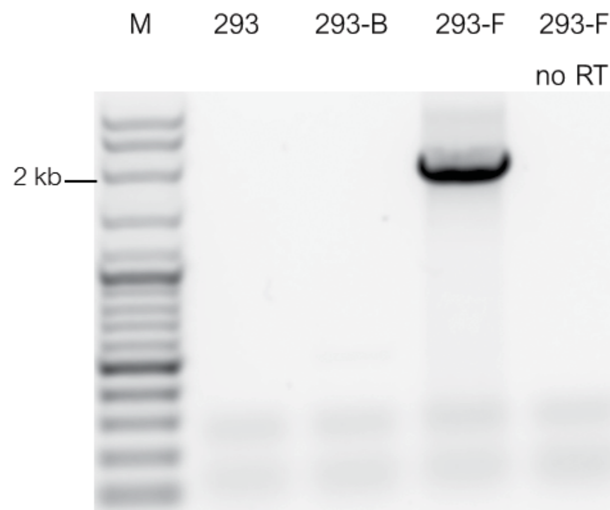

Figure 3. Electrophoresis of FimA-PVXCPRT-PCR product (1.9 kb) of RNA extracted from HEK293 cells transfected with pcDNA3.Fim-PVXCP (293-F) or pcDNA3.BCL1scfv-PVXCP (293-B) or no transfection (293). No RT indicates RT PCR without reverse transcriptase. M:VC 100 bp Plus DNA ladder

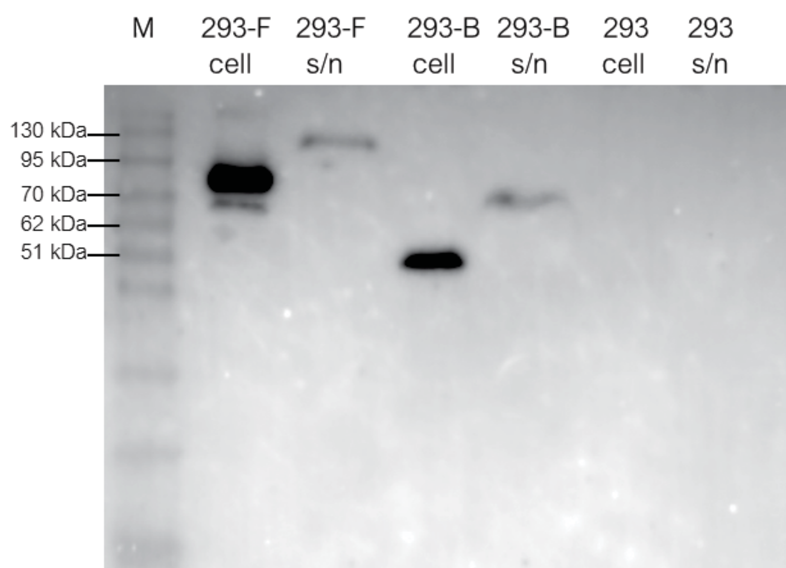

Figure 4. Detection of FimA-PVXCP protein by Western blot analysis.Proteins remained inside the cells (cell) and in the medium (s/n) of HEK293 cellstransfected with pcDNA3.Fim-PVXCP (293-F) or pcDNA3.BCL1scfv-PVXCP (293-B) or no transfection (293) were analyzed. M: Chromatein Prestained Protein Ladder, vivantis.

Discussion

The constructed pcDNA3.FimA-PVXCP DNA vaccine was designed in the way that it will be secreted from the cells. As the protein was destined into secretory pathway, in the endoplasmic reticulum (ER) the protein will be post-translational modified by, for example, glycosylation. When amino acid sequence of FimA-PVXCP was analyzed using NetNGlyc 1.0 server [15] and NetOGlyc 4.0 server [16], the data showed that FimA-PVXCP contains many potential N- and O- glycosylation sites. FimA-PVXCP might be glycosylated and therefore the size of the secreted protein was larger than the protein remain inside the cells.

Protein expressed from this DNA vaccine included full-length FimA typeII which may contains many antigenic determinants. It has been shown that anti-FimA type II antisera could react with strains with other FimA types [17]. Therefore, this DNA vaccine might be also able to induce immunity against other *P. gingivalis* FimA types.

Determination of FimA-PVXCP expression in mammalian cells is just the initial step in DNA vaccine production. To examine the ability of the DNA vaccine to induce protective immunity, the vaccine will be further administered into animal models [13]. DNA vaccines using PVXCP fusion in pcDNA3 backbone have been shown to induce high levels of antibody and protection against specific antigen [18]. Therefore this FimA-PVXCP DNA vaccine is expected to induce high antibody level as well.

Conclusion

FimA-PVXCP DNA vaccine was able to be expressed in HEK293 cells as confirmed by RT-PCR and Western blot analysis.

Acknowledgements

Plasmid pcDNA3.BCL1scfv.PVXCP and anti-PVXCP antibody were provided by Dr Natalia Savelyeva,Cancer Sciences Unit, Faculty of Medicine, University of Southampton. We thank Assistant Professor Dr Sarawut Kumphune, Faculty of Allied Health Sciences, Naresuan University, for kindly provided HEK293 cells. We also would like to thank Dr Paiboon Jitprasertwong, Faculty of Dentistry, Naresuan University, for sharing HRP-conjugated goat anti-rabbit IgG. This study was funded by Naresuan Universiy.

References

1. Amano A, Nakagawa I, Kataoka K, Morisaki I Hamada S. Distribution of *Porphyromonas gingivalis* strains with FimA genotypes in periodontitis patients. J Clin microbiol 2000; 37(5): 1426-1430.

2. Missailidis C, Umeda J, Ota-Tsuzuki C, Anzai D Mayer M. Distribution of fimA genotypes of *Porphyromonas gingivalis* in subjects with various periodontal conditions. Oral Microbiol Immun 2004; 19: 224-229.

3. Dye B. Global periodontal disease epidemiology. Periodontol 2000 2012; 58: 10-25.

4. Chaisupamongkollarp S, Jaturanon S, Subhakorn S Ploysangngam P. Caries and periodontal experience among 998 priests and novices in Bangkok. J Med Assoc Thai 2008; 91: s130-138.

5. Chiu B. Multiple infections in carotid atherosclerotic plaques. Am Heart J 1999; 138: S534-S536.

6. Haraszthy V, Zambon J, Trevisan M, Zeid M Genco R. Identification of periodontal pathogens in atheromatous plaques. J Periodontol 2000; 71: 1554-1560.

7. Meurman J, Sanz M Janket S. Oral health, atherosclerosis, and cardiovascular disease. Crit Rev Oral Biol Med 2004; 15: 403-413.

8. Seymour G, Ford P, Cullinan M, Leishman S Yamazaki K. Relationship between periodontal infections and systemic disease. Clin Microbiol Infect 2007; 13(suppl 4): 3-10.

9. Nakano K, Inaba H, Nomura R, Nemoto H, Takeuchi H, Yoshioka H, et al. Distribution of *Porphyromonas gingivalis* fimA genotypes in cardiovascular specimens from Japanese patients. Oral Microbiol Immun 2008; 23: 170-172.

10. Persson G. Rheumatoid arthritis and periodontitis - inflammatory and infectious connections. Review of the literature. J Oral Microbiol 2012; 4:(16 screens). Available from: URL:http://dx.doi.org/10.3402%2Fjom.v4i0.11829.

11. Terpenning M, Taylor G, Lopatin D, Kerr C, Dominguez B Loesche W. Aspiration pneumonia: dental and oral risk factors in an older veteran population. J Am Geriatr Soc 2001; 49(5): 557-563.

12. Kawabata S, Terao Y, Fujiwara T, Nakagawa I Hamada S. Targeted salivary gland immunization with plasmid DNA elicits specific salivary immunoglobulin A and G antibodies and serum immunoglobulin G antibodies in mice. Infect Immun 1999; 67(11): 5863-5868.

13. Yu F, Xu Q Chen W. A targeted fimA DNA vaccine prevents alveolar bone loss in mice after intra-nasal administration. J Clin Periodontol 2011; 38: 334-340.

14. Savelyeva N, King C, Vitetta E Stevenson F. Inhibition of a vaccine-induced anti-tumor B cell response by soluble protein antigen in the absence of continuing T cell help. Proc Natl Acad Sci U S A 2005; 102(31): 10987-10992.

15. Gupta R, E. J Brunak S, Prediction of N-glycosylation sites in human proteins.In preparation 2004. Available from: URL: http://www.cbs.dtu.dk/services/NetNGlyc/

16. Steentoft C, Vakhrushev S, Joshi H, Kong Y, Vester-Christensen M, Schjoldager K, et al. Precision mapping of the human O-GalNAc glycoproteome through SimpleCell technology. EMBO J 2013; 32(10): 1478-88.

17. Nagano K, Abiko Y, Yoshida Y Yoshimura F. Genetic and antigenic analyses of *Porphyromonas gingivalis* FimA fimbriae. Mol Oral Microbiol 2013; 28(5): 392-403.

18. Savelyeva N, Munday R, Spellerberg M, Lomonossoff G Stevenson F. Plant viral genes in DNA idiotypic vaccines activate linked CD4+ T-cell mediated immunity against B-cell malignancies. Nat Biotechnol 2001; 19(8): 760-764.

Corresponding author:
Dr. Jantipa Jobsri
Faculty of Dentistry, Naresuan University,
Phitsanulok, Thailand.
Tel. 085-814-9219
E-mail : jantipaj@nu.ac.th