The EuroBiotech Journal
RESEARCH ARTICLE
Plant Biotechnology

Structure of PAP-IgM FcK fusion protein with J-chain expressed in transgenic plant

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Abstract

Transgenic plants expressing immunoglobulin (Ig) M Fc-fused Prostate acid phosphatase (PAP) antigenic proteins (PAP-IgM FcK) and J-chain proteins were generated by Agrobacterium-mediated transformation. The Fc region was tagged with the ER retention motif (KDEL) to make PAP-IgM FcK. Two transgenic plants were crossed together to generate F_1 expressing both PAP-IgM FcK and J-chain proteins (PAP-IgM FcK × J-chain). PCR and RT-PCR analyses confirmed the transgene insertion and mRNA transcription of PAP-IgM FcK and J-chain in leaf tissue of PAP-IgM FcK × J-chain F_1 plant. Western blot confirmed the expression of PAP-IgM FcK × J-chain protein. Size exclusion (SEC)-high performance liquid chromatography (HPLC) and Bio-transmission electron microscope (TEM) analyses were performed to show the size and shape of the PAP- IgM FcK × J-chain fusion proteins. These results suggest that PAP-IgM FcK with J-chain can be produced in plant expression system with plant crossing.

Keywords: Transgene, Plant Biotechnology, Immunoglobulin, Protein Engineering

Introduction

Mammal cells can improve protein bioactivity by post-translational modifications, and thus recombinant proteins are produced mainly in mammalian cells. Most of the recombinant proteins approved by the FDA so far are produced in mammalian cells (Lai et al., 2013;Rasooly et al., 2013;Linderholm and Chamow, 2014). Transgenic systems such as yeast, bacteria and insect cell lines are available for recombinant therapeutic protein production as well. However, these systems have various disadvantages such as production cost, production time, and contamination risk (Shanmugaraj and Ramalingam, 2014). On the other hand, plant molecular farming is emerging as a potential for expression system, due to its economical, safety, and practical advantages. In this paper, the recombinant PAP-Fc was produced by fusing PAP to the Fc fragment of human immunoglobulin M in transgenic plants. Transgenic plant expressing PAP-IgM FcK and transgenic plant expressing J-chain were cross-pollinated to express and assemble polymers.

Prostate cancer is a malignant disease that occurs in males worldwide. In prostate tissues, including both the normal prostate and prostate cancer, the expression of several specific proteins, such as prostatic acid phosphatase, prostate-specific membrane antigen (PSMA), and prostate-specific antigen (PSA) has been reported (Fong et al., 1997;Fong et al., 2001;Roos et al., 2005;Fujio et al., 2015). Prostatic acid phosphatase is a secretory prostate-specific protein with an estimated molecular mass of 45 kDa. PAP is overexpressed in 95% of prostate cancer tissue, and the tissue specificity of PAP is made a target antigen for immunotherapy against prostate cancer (Peshwa et al., 1998;Saif et al., 2014). Several vaccine candidates have been developed based on the PAP protein such as cell-based medicine (Kantoff et al., 2010;Di Lorenzo et al., 2012), DNA vaccines (McNeel et al., 2009), or peptide antigen (Machlenkin et al., 2005;Matsueda et al., 2005).

In general, Fc-fusion protein is used by fusing immunoglobulin G Fc fragment with antigenic proteins for virus and cancer (Van den Brande et al., 2003;Strober and Me-

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DOI: 10.2478/ebtj-2023-0006

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non, 2007;Cines et al., 2008;Kuter et al., 2008;Korhonen and Moilanen, 2009;Peters et al., 2010;Powell et al., 2012;Linderholm and Chamow, 2014).

In a previous study, prostate acid phosphatase, an antigenic protein for prostate cancer, is produced in plants by polymer proteins such as dimers and hexamers, which were fused to immunoglobulin G Fc fragment but immunoglobulin M Fc fragment. Immunoglobulin M is formed as covalent polymers structure of the H2L2 monomer subunit (Milstein et al., 1975;Sorensen et al., 1999). IgM forms mainly pentamers. In the pentamer IgM, the Fc regions of the monomer are linked end to end through the disulfide bonds of the J chain of a 15 kDa protein covalently (Krugmann et al., 1997;Sorensen et al., 1999;Woof and Russell, 2011). The J-chain is associated with formation of pentameric IgM through the disulfide binds involving the cysteine residue in the tailpiece of μ heavy chain. In addition, J-chain plays an important role in intracellular assembly of the IgM by modulating their structure, and thereby regulates the effector function (Brandtzaeg and Prydz, 1984;Vaerman et al., 1998). The polymer structures such as IgM increase the avidity of antibodies to antigens and are required for functions such as complement activation and epithelial secretion. Thus, basic knowledge about the structure for the polymerization will provide information on the regulation of essential functions of IgM. Also, it can be useful for construction of the polymer of the engineered antibody-derived molecule as a means to increase modulation features such as ligand avidity, tissue penetration, and serum half-life (Sorensen et al., 1999).

In this study, the transgenic plants were obtained to produce the PAP-IgM FcK × J-chain for prostate cancer vaccine, and its structure was investigated.

Materials & Methods

Generation of F_1 plant expressing both PAP-IgM FcK and J-chain

The transgenic tobacco plant lines expressing PAP-IgM FcK (Kang et al., 2017) and J-chain, respectively, were grown to produce flowers in the greenhouse. Before the stamen releases the pollen, stamens from transgenic plants expressing PAP-IgM FcK were collected in vial and released their pollen. And then, pollens of PAP-IgM FcK transferred to the stigma of transgenic plant expressing J-chain (Fig. 1). The F_1 seeds (PAP-IgM FcK × J-chain) were collected from the cross-pollinated plants and sterilized in the seed disinfecting solution including 15% commercial bleach (Yuhan Clorox, Seoul, Korea) and 20% ethanol. The disinfected seeds were washed three times with autoclaved deionized water and placed on Murashige and Skoog (MS) medium [30 g•L^{-1} of Sucrose, 8 g•L^{-1} of Plant agar, and 4.8 g•L^{-1} of MS B5 vitamin (Duchefa Biochemie, Haarlem, Netherlands)] containing 100 mg•L^{-1} kanamycin. Transgenic seeds were germinated at 25°C under a long-day photoperiod condition (16 h day/8 h night). Non-transgenic seeds were germinated on MS medium without kanamycin and used as a negative control.

Polymerase Chain Reaction (PCR) amplification from genomic DNA of F_1 plant

Genomic DNAs of transgenic and non-transgenic plants were isolated from 100 mg tobacco leaves using a DNA extraction kit (RBC Bioscience, Seoul, South Korea). Genomic DNA isolated from transgenic plants was PCR amplified using Mastercycler® gradient (Eppendorf, Hamburg, Germany) to confirm the presence of PAP-IgM FcK (2,220 bp), and J-chain (180 bp) recombinant genes using the following forward and reverse primer pairs: for PAP-IgM FcK × J-chain (forward primer 5'-CCT ACT CTG GCA GCC CAT C-3', reverse primer 5'-TCA GAG TTC ATC TTT GTA GCA G-3'); for J-chain fragment (forward primer 5'-GAG TAG CTG GGA TTA CAG CC-3', reverse primer 5'- GGA CCA CAG CTG TGT AGC AC-3'). The PCR conditions were as follows; for PAP-IgM FcK × J-chain initial denaturation at 94°C for 120 s; 29 cycle of denaturation at 94°C for 20 s, annealing of 59.5°C (For IgM FcK and J-chain fragment), 63°C (For PAP fragment) for 20 s, extension at 72°C for 26 s (For J-chain fragment), 72 s (For PAP fragment), 92 s (For IgM FcK fragment), respectively, and final extension at 72°C for 120 s. A non-transgenic tobacco plant was used as a negative control, while the PAP-IgM FcK and J-chain transgenic plants were used as a positive control.

RNA isolation from F_1 plant leaf and Reverse Transcription-Polymerase Chain Reaction (RT-PCR) amplification

RT-PCR analysis identified complementary DNA (cDNA) of PAP-IgM FcK × J-chain isolated from F_1 plantlet. Total RNA of PAP-IgM FcK × J-chain transgenic crossing plant was extracted from F_1 transgenic plant leaf samples using the RNeasy Plant Mini kit (Qiagen, Vaencia, CA). Isolated total RNA samples from plantlet removal and cDNA synthesis were performed with an experiment using the QuantiTect Reverse Transcription kit (Qiagen, Valencia, CA). Each cDNA sample of PAP-IgM FcK × J-chain was used as a template for the RT-PCR analysis. RT-PCR was identified using the maxime PCR premix kit (Intron Biotechnology, Seoul, South Korea). Synthesized cDNA was PCR amplified to confirm the presence or absence of PAP-IgM FcK × J-chain recombinant genes using the following forward and reverse primer pairs: for PAP fragment of PAP-IgM FcK × J-chain (forward primer 5'-CCT ACT CTG GCA GCC CAT C-3', reverse primer 5'-GTC CTG GCC ATG TAA TCC TG-3'); for IgM Fc fragment of PAP-IgM FcK × J-chain. (forward primer 5'-GCT GAG GCC AAA GAG TCT G-3', reverse primer 5'-GCA TCC ACT GCA CGA AGA C-3'); for KDEL fragment of PAP-IgM FcK × J-chain (forward primer 5'-GTA CTT CGC CCA CAG CAT C-3', reverse primer 5'-TCA GAG TTC ATC TTT GTA GCA G-3'); for J-chain fragment of PAP-IgM FcK × J-chain (forward primer 5'-GAG TAG CTG GGA TTA CAG CC-3', reverse primer 5'-GGA CCA CAG CTG TGT AGC AC-3'). The RT-PCR conditions were as follows; initial denaturation at 94°C for 120 s; 30 cycle of denaturation at 94°C for 20 s, annealing of 58°C for 10 s, extension at 72°C for 42 s, and final extension at 72°C for 120 s. A non-transgenic plant

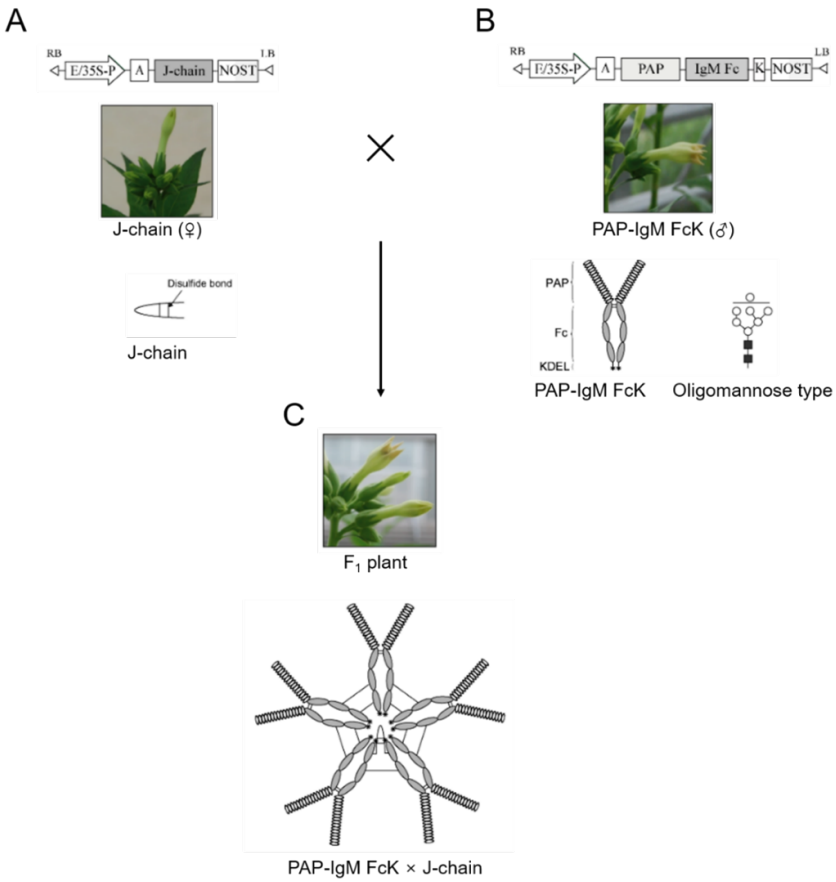


Figure 1. Schematic diagram of the plant expression vector and crossing transgenic plant. PAP-IgM FcK and J-chain genes in plant expression cassettes were designed for plant expression vectors pBI PAP-IgM FcK and pBI J-chain, repectively. The PAP-IgM FcK transgenic plant (A) was cross-pollinated with the J-chain transgenic plant (B), and F_1 transgenic plant expressing both proteins were obtained (C). The gene expression cassette had E/35S-P, cauliflower mosaic virus 35S promoter with duplicated upstream B domain for expression of PAP-IgM FcK recombinant protein. A, untranslated leader sequence of alfalfa mosaic virus RNA4; NOST, terminator of nopaline synthase gene; K, KDEL peptide sequence, the endoplasmic reticulum (ER) retention signal. The recombinant protein and glycan structures are expected from the PAP-IgM FcK and J-chain respectively. Expected glycan structure: the symbols of the glycan structures are as follows: GlcNAc, the black square; mannose, white circle.

and transgenic plants of PAP-IgM FcK and J-chain were used as negative and positive controls, respectively. The elongation factor 1-α (EF-1α) gene, which is involved in plant growth, was used as a reference gene.

Immunoblot analysis

Immunoblot analysis was performed to determine the presence of protein expression of PAP-IgM FcK × J-chain in F_1 transgenic plant. The leaf (100 mg) was homogenized in 300 µL 1× PBS (7 mM NaCl, 0.135 mM KCl, 0.325 mM $Na_2HPO_4•2H_2O$, and KH_2PO_4 0.075 mM) to extract the total soluble proteins. A volume of 20 µL of extracted plantlet samples (100 mg of leaf/300 µL) was mixed with 5 µL of 5× protein loading dye (1 M Tris-HCl, 50 % glycerol, 10% SDS, 5% 2-mercaptoethanol, 0.1% bromophenol blue). The leaf samples of F_1 transgenic plants loaded in 10% polyacrylamide gel and transferred to a nitrocellulose membrane (NC membrane) (Millipore, Billerica, MA). Transferred NC membranes were blocked with 5% skim milk (Sigma Aldrich, St.Louis, MO) in 1× TBS-T buffer [0.05% (v/v) Tween 20 in 1× TBS (150 mM NaCl, 50 mM Tris-HCl, pH 7.4)] for 2 hr at room temperature (RT). Transferred NC membranes were incubated for 2 hr at RT with either rabbit anti-human PAP antibody (Abcam, Cambridge, MA) diluted in blocking buffer at 1: 5,000, mouse anti-human IgM Fc conjugated to horseradish peroxidase (Abcam, Cambridge, MA) diluted in blocking buffer at 1: 5,000. And then, rabbit anti-human PAP antibody treated NC membrane was incubated for 2 hr at RT with secondary antibody goat anti-rabbit IgG antibody conjugated to horseradish peroxidase (Bethyl Laboratories, Montgomery, TX) diluted in blocking buffer at 1: 5,000. The protein bands of transgenic plants were visualized by the exposing membrane to an x-ray film (Fuji, Tokyo, Japan) using the Clarity Western enhanced chemiluminescence substrate (Bio-Rad Hercules CA). Non-transgenic tobacco plant, the human recombinant PAP (Sino Biological, Beijing, China), and PAP-IgM FcK transgenic plant were used as negative and positive controls, respectively.

Purification of recombinant PAP-IgM FcK × J-chain protein from transgenic plant leaves

Recombinant PAP-IgM FcK × J-chain fusion protein was purified according to a previous study (Kim et al., 2021;Lim et al., 2022). Briefly, the homogenized plant leaves of extraction buffer were centrifuged at 8,800 ×g for 30 min at 4°C. The suspension was filtered through a Miracloth (Merck Millipore Corporation, Darmstadt, Germany), and it was adjusted to pH 5.1 using the acetic acid glacial (Duksan, Seoul, Korea). The suspension was centrifuged at 10,200 × g for 30 min at 4°C and neutralized again at pH 7.0. And then, ammonium sulfate was added to 8 and 40%, respectively. After the saturated suspension was incubated for overnight at 4°C. The suspension was centrifuged at 8,800 ×g for 30 min at 4°C, the pellet was re-suspended in 1× PBS to 1/10 of the previous volume, and the final suspension was then centrifuged at 10,200 ×g for 30 min at 4°C (Kim et al., 2021;Lim et al., 2022). The supernatant was then filtered through a hydrophilic PVDF 0.45 μm filter (Millipore, Billerica, MA). The recombinant PAP-IgM FcK × J-chain fusion protein was purified using Capture select™ IgA Affinity Matrix (Thermo Fisher Scientific, Waltham, MA), POROS™ CaptureSelect™ IgM Affinity Materix (Thermo Fisher Scientific, Waltham, MA), respectively. Elutes of plant-derived PAP-IgM FcK × J-chain recombinant proteins were dialyzed against 1 × PBS (pH 7.4) buffer. The protein concentration was determined a using an Epoch spectrometer (UV-Vis Microplate Spectrophotometer, Biotech, Winooski, VT), and purified proteins were visualized by SDS-PAGE using 10% acrylamide gel.

Size-Exclusion Chromatography-High-Performance Liquid Chromatography (SEC-HPLC) analysis of the recombinant fusion protein purified from F_1 plants

Size-Exclusion Chromatography-High-Performance Liquid Chromatography (SEC-HPLC) analysis was performed to determine the protein size of the recombinant PAP-IgM FcK × J-chain fusion proteins. The protein size of the recombinant PAP-IgM FcK × J-chain fusion proteins measured using an Agilent 1260 Infinity Quaternary Bio-inert instrument system (Agilent Technologies Inc., Santa Clara, CA) and an Agilent Bio SEC-5μm 300Å column, 7.8 x 300 mm (Agilent Technologies Inc., Santa Clara, CA). The HPLC system and SEC column were equilibrated in 150 mM sodium phosphate, pH 7.0 to a flow rate of 1 mL/min at 25°C. The recombinant PAP-IgM FcK × J-chain fusion proteins were detected by monitoring absorbance at 280 nm. AdvanceBio SEC 300Å protein standard, lyophilized (Agilent Technologies Inc., Santa Clara, CA) was used as a standard. Separation of recombinant fusion proteins was achieved in 1× PBS at a flow rate of 1 mL/min. And then, to obtain a high concentration of fractionated recombinant proteins, it was concentrated using the Amicon Ultra centrifugal filter (Millipore, Billerica, MA).

Bio-Transmission Electron Microscopy (Bio-TEM) analysis

The purified PAP-IgM FcK × J-chain fusion proteins were analyzed to determine its protein structure. Negative staining of plant-derived recombinant fusion proteins was treated according to the Korea Basic Science Institute protocol. Briefly, 5 μL protein samples of PAP-IgM FcK × J-chain were applied to glow-discharged carbon-coated copper grids. After allowing the samples to absorb for 2 min and blotting off buffer solution onto Whatman filter paper (Sigma Aldrich, St.Louis, MO), then the samples on the grids were stained with 2% (w/v) uranyl acetate (UrAc) for 1 min. And then, it was blotted off UrAc. The plant-derived recombinant PAP-IgM FcK × J-chain fusion protein samples were visualized using a Tecnai G2 Spirit Twin transmission electron microscope fitted with anti-contaminator (FEI, Hillsboro, OR, USA, instrument at the Korea Basic Science Institute) operated at 120 kV. Images were recorded with a 4K94K, Ultrascan 895 CCD camera (Gatan Inc., Pleasanton. CA) at a magnification of 52,000.

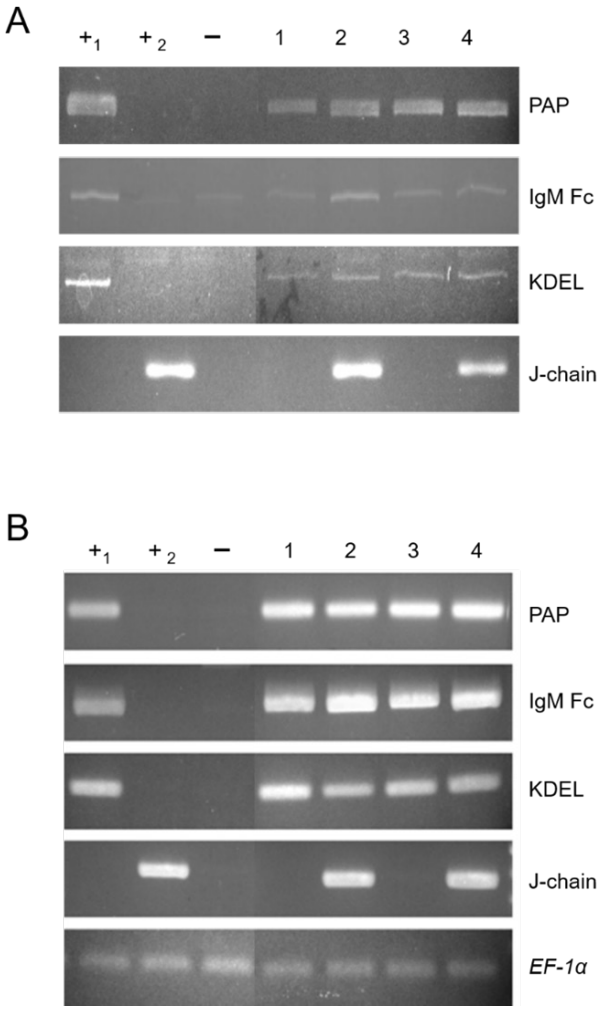
Results

Generation of transgenic tobacco plants expressing PAP-IgM FcK × J-chain in F_1 plants through cross-fertilization

The F_1 seeds were obtained from cross-pollination between the transgenic tobacco line expressing PAP-IgM FcK and the transgenic tobacco line expressing J-chain, respectively (Fig. 1). The PCR analysis was performed to confirm the presence of PAP-IgM FcK × J-chain genes isolated from F_1 transgenic tobacco plant leaf obtained through cross pollination, respectively (Fig. 2). The F_1 plants were randomly selected (Fig. 2A). The primers for PAP, IgM Fc, KDEL, and J-chain sequences were used for PCR, respectively. Three F_1 transgenic plants were randomly selected for semi-quantitative RT-PCR to confirm the mRNA expression of PAP, IgM Fc fragment, KDEL, and J-chain of PAP-IgM FcK × J-chain (Fig. 2 B). RT-PCR products of the expected sizes of PAP, IgM Fc fragment, KDEL, and J-chain sequences were detected in cDNA isolated from transgenic plant leaf (Fig. 2 B). The PAP-IgM FcK × J-chain transgene was not detected in non-transgenic plant (Fig. 2). The EF-1α band was used as a standard control and was detected at 67 bp.

Expression of PAP-IgM FcK × J-chain in F_1 transgenic plants

F_1 transgenic tobacco plants were applied to extract total soluble proteins, and their transgene expression was analyzed by Immunoblotting (Fig. 3). F_1 transgenic lines with PAP-IgM FcK × J-chain showed an approximately 83 kDa (Fig. 3) protein bands. Commercial PAP and transgenic plant leaf expressing PAP-IgM FcK were used as a positive control, respectively. When PAP-IgM FcK × J-chain F_1 transgenic plant leaf samples were treated with anti-human PAP antibody, 45 kDa protein band was detected for other than 83 kDa protein band, respectively (Fig. 3B anti-PAP). In PAP-IgM FcK × J-chain F_1 plant, IgM Fc antibody protein bands were detected at 71 kDa and more than 240 kDa (Fig. 3B anti-IgM Fc). No band was observed in non-transgenic plant (Fig. 3).



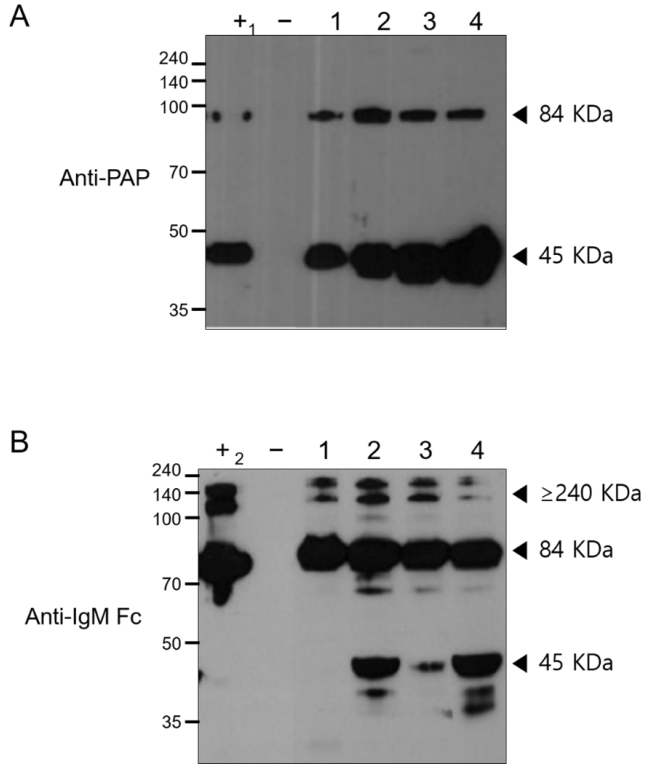


Figure 2. PCR and RT-PCR analyses of PAP-IgM FcK × J-chain gene expressed in F_1 transgenic tobacco plant. Genomic DNA and total RNA extracted from tobacco leaf tissues were used as template in PCR and RT-PCR with PAP, IgM Fc, KDEL, and J-chain specific primers to amplify a portion of PAP-IgM FcK × J-chain genes. Samples were analyzed by electrophoresis through 1 and 3% (wt/vol) agarose gels, respectively. Gels were stained with ethidium bromide and DNA was visualized under UV. (A) PCR analysis of PAP-IgM FcK (2,220 bp) and J-chain (540 bp) genomic DNA isolated from transgenic plant leaf. +2, positive control (E.coli derived J-chain); +1, positive control (*E.coli* derived PAP-IgM FcK). (B) Reverse transcription (RT)-PCR analysis of PAP-IgM FcK and J-chain. Total mRNA isolated from plant leaves of randomly selected PAP-IgM FcK and J-chain transgenic plants. +2, positive control (Plant derived J-chain); +1, positive control (Plant derived PAP-IgM FcK).

Purification of plant-derived PAP-IgM FcK × J-chain fusion proteins

The PAP-IgM FcK × J chain fusion protein was purified appropriately from the F_1 transgenic tobacco plant leaves. SDS-PAGE showed that the PAP-IgM FcK × J-chain had the expected recombinant fusion protein size (Fig. 4).

Figure 3. Immunoblot analysis of PAP-IgM FcK × J-chain transgenic plant. Immunoblot analysis of F_1 plants using the specific antibodies conjugated to horseradish peroxidase. The total soluble proteins were extracted from tobacco leaf of randomly selected PAP-IgM FcK × J-chain transgenic plants. The bands for PAP-IgM FcK (83.3 kDa) were detected with HRP-conjugated rabbit anti-human PAP antibody (1: 5,000) (A) and HRP-conjugated goat anti-human IgM Fc antibody (1: 5,000) (B), respectively. $+_1$, positive control (mammalian-derived recombinant PAP, 50 ng); $+_2$, positive control (PAP-IgM FcK extracted from plant leaves, 20 µL)

Size-Exclusion Chromatography-High Performance Liquid Chromatography (SEC-HPLC) analysis of plant-derived PAP-IgM FcK × J-chain proteins

SEC-HPLC was performed to identify the size distribution of the PAP-IgM FcK × J-chain fusion protein. The recombinant PAP-IgM FcK × J-chain protein was migrated on HPLC as peaks between 5 and 8 min (Fig. 5).The peaks were correspond to more than 150 kDa, 300 kDa, and similar 1,500 kDa, which predicted molecular weight of monomer (M), dimer, trimer (D/T), and polymer (P). On HPLC, corresponding to a molecular weight greater than 670 kDa. Interestingly, PAP-IgM FcK × J-chain protein migrated about 30 s (Fig. 5).

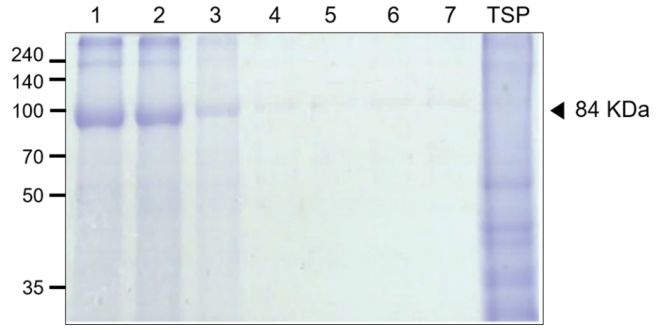


Figure 4. SDS-PAGE analysis of PAP-IgM FcK × J-chain recombinant proteins purified from F_1 transgenic plants. Purified recombinant proteins (F1 to F7, Fraction protein sample numbers) were loaded onto 10% acrylamide gels and stained with coomassie brilliant blue R250. F.T, flow through. Each fraction sample was loaded with 10 µL.

Visualization of plant-derived PAP-IgM FcK × J-chain fusion proteins by Bio-transmission electron microscopy (Bio-TEM)

Bio-Transmission Electron Microscopy (Bio-TEM) was performed to visualize the shape of the plant-derived PAP-IgM FcK × J-chain fusion proteins. EM with negative stained PAP-IgM FcK × J-chain showed substantial structure (Fig. 6). The recombinant PAP-IgM FcK × J chain proteins were observed as a complex form. In particular, the pentamer shape was observed in the plant-derived PAP-IgM FcK × J-chain fusion proteins (Fig. 6). The difference in shape may be caused by the orientation of the plant-derived PAP-IgM FcK × J-chain proteins bound to the chip.

Discussion

In this study, F_1 transgenic plants expressing PAP-IgM FcK × J-chain proteins as a prostate cancer vaccine candidates were generated with crossing between transgenic plants expressing PAP-IgM FcK and J-chain proteins.

PAP-IgM FcK × J-chain F_1 transgenic plants were produced through the cross-pollination of the IgM FcK transgenic plants with the J-chain transgenic plant. Both PCR and RT-PCR analyses confirmed that all randomly tested F_1 transgenic plants expressing the PAP-IgM FcK × J-chain transgenes had the

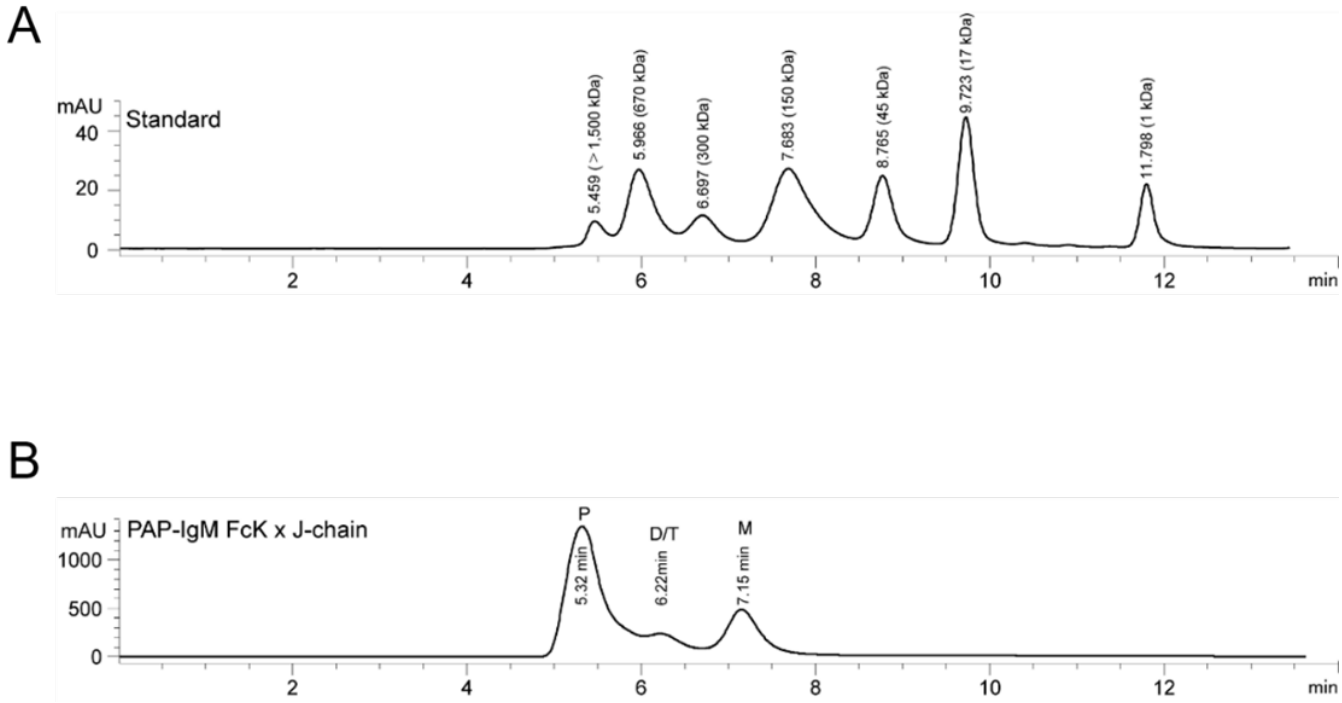


Figure 5. SEC-HPLC chromatogram of plant-derived recombinant PAP-IgM FcK × J-chain proteins from F_1 transgenic plant. SEC-HPLC chromatogram was performed to determine the protein size of the recombinant PAP-IgM FcK × J-chain purified from F_1 transgenic plant. Indicated retention times were used to estimate the molecular weights of recombinant protein based on AdvanceBio SEC 300Å Protein Standard.

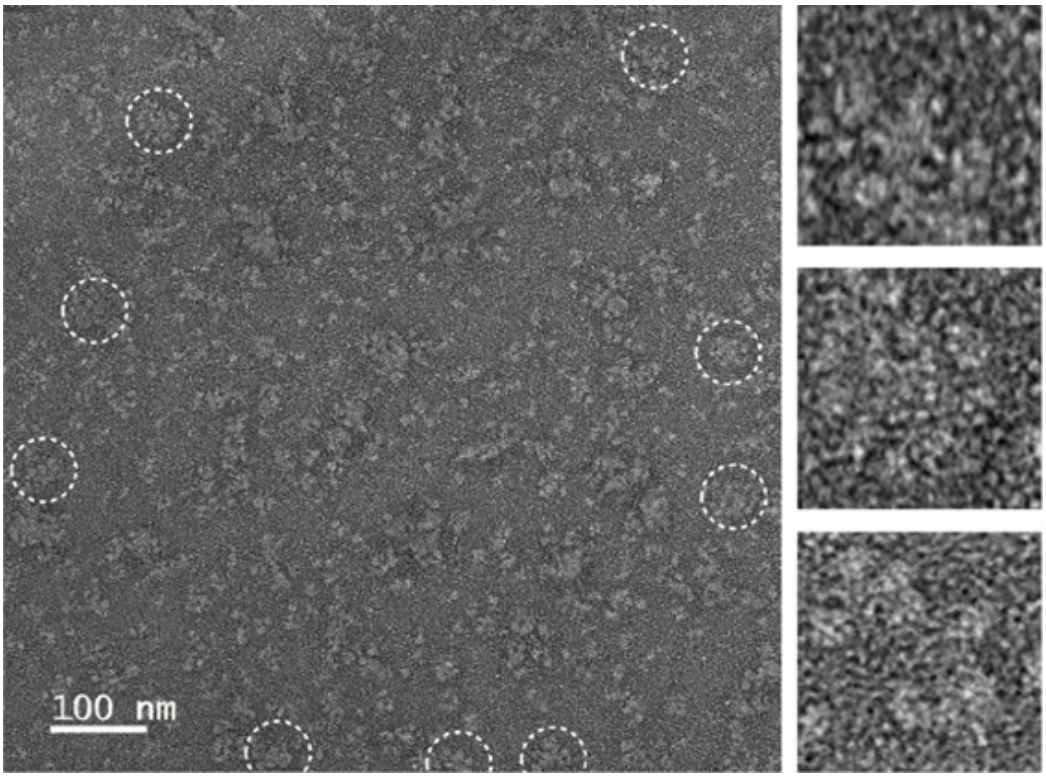


Figure 6. Bio-TEM image of recombinant PAP-IgM FcK × J-chain proteins from F_1 transgenic plant. Bio-TEM performed to analyze protein structure of PAP-IgM FcK × J-chain proteins. The white dotted circle represents the plant-derived recombinant PAP-IgM FcK × J-chain proteins. PAP-IgM FcK × J-chain proteins showed polymeric structures.

PAP-IgM FcK and J-chain transgenes, and their transcripts. Immunoblot analysis confirmed expression of the recombinant fusion proteins in F_1 transgenic plants. All F_1 transgenic plants had strong PAP-IgM FcK protein expression. The J-chain DNA presence was confirmed in PCR and RT-PCR analyses. The results showed that the plant expression system can simultaneously express two proteins in a F_1 plant through cross-pollination. The leaves of transgenic plants were used to purify the PAP-IgM FcK× J-chain fusion proteins to confirm the protein size and structure.

The polymeric pentamer IgM antibodies are unique since they contain small polypeptides known J-chains, including heavy and light chains. The J-chain provides pentameric IgM with the capacity to bind the polymeric immunoglobulin receptor, and it regulates the polymeric immunoglobulin structure. The J-chain forms with pentameric IgM through a disulfide bond with IgM, which is associated with the penultimate cysteine residue in the secretory tailpiece of the μ or the α heavy chain (Sorensen et al., 1999;Johansen et al., 2000;Hiramoto et al., 2018). For this reason, to take advantage of the role of these J-chains, the PAP-IgM FcK × J-chain recombinant proteins were produced by cross-pollination to the J-chain expressing transgenic plants. And then, SEC-HPLC and Bio-TEM were conducted to measure the size and shape of the plant-derived PAP-IgM FcK × J-chain proteins. The sizes of plan-derived PAP-IgM FcK × J-chain proteins were measured using SEC-HPLC analyses. Plant-derived PAP-IgM FcK × J-chain fusion proteins existed in various forms similar to hexamer. In nature, IgM exists as a pentamer, and occasionally as a hexamer, which lacks a J-chain (Randall et al., 1992;Wiersma et al., 1998). The protein size was different depending on the presence of J-chain in PAP-IgM FcK. PAP-IgM FcK showed both the pentamer/hexamer size and the PAP-IgM FcK × J-chain only are supposed to show the pentamer size. Bio-TEM was performed to visualize the hexamer or pentamer shapes of the plant-derived PAP-IgM FcK × J-chain fusion proteins. Such polymeric fusion protein shape were observed in the plant-derived PAP-IgM FcK × J-chain fusion protein (Fig. 6). While previous studies showed clear differences in the structural form of IgM (Roux, 1999;Hiramoto et al., 2018)(Roux, 1999, Hiramoto et al., 2018), there has been no noticeable difference in protein shape in this study.

In conclusion, in this paper, we reported that the plant cross-pollination of transgenic plants expressing PAP-IgM FcK and J-chain proteins could potentially be applied to generate PAP-IgM FcK × J-chain proteins as a immunetherapeutic prostate cancer vaccine.

Acknowledgments

This research was supported by the National Research Foundation of Korea grant [2021R1F1A1063869], Basic Science Research Program through the National Research Foundation

of Korea (NRF) funded by the Ministry of Education [NRF-2020R1I1A1A01072021], and the Bio & Medical Technology Development Program of the National Research Foundation (NRF) and funded by the Korean government (MSIT) (No.2019M3E5D5067214).

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