Amino Acids (2012) 42:2215–2222
DOI 10.1007/s00726-011-0961-8

ORIGINAL ARTICLE

A bursal pentapeptide (BPP-I), a novel bursal-derived peptide, exhibits antiproliferation of tumor cell and immunomodulator activity

Xiu L. Feng · Qing T. Liu · Rui B. Cao · Bin Zhou · Fang Q. Wang · Wen L. Deng · Ya F. Qiu · Yu Zhang · Hassan Ishag · Zhi Y. Ma · Qi S. Zheng · Pu Y. Chen

Received: 3 April 2011 / Accepted: 8 June 2011 / Published online: 13 July 2011
© Springer-Verlag 2011

Abstract The bursa of Fabricius (BF) is the central humoral immune organ unique to birds. Here, we isolated a novel bursal pentapeptide I (BPP-I), LGPGP, from BF. BPP-I could play inhibition effect on MCF-7 but not on CEF or Vero cell proliferation in vitro, and enhance antitumor factor p53 protein expression. Also, BPP-I stimulated antibody production in a dose-dependent manner in hybridoma cell. Furthermore, BPP-I could induce various immune responses in mice immunization experiments, including increase antibody production and cytokines IL-4 and IFN-γ level, and induce T-cell immunophenotyping. These results suggest that BPP-I is a potential immunomodulator of antitumor and immunity. The study could provide some novel insights on the probable candidate reagent for the antitumor and immune improvement.

X. L. Feng · R. B. Cao · B. Zhou · F. Q. Wang · W. L. Deng · Y. Zhang · H. Ishag · P. Y. Chen (✉)
Division of Key Lab of Animal Disease Diagnosis and Immunology of China's Department of Agriculture, College of Veterinary Medicine, Nanjing Agriculture University, Nanjing 210095, People's Republic of China
e-mail: puyanchen@yahoo.com.cn

Q. T. Liu
Nanjing Tianbang Bio-Industry Co. Ltd, Nanjing, People's Republic of China

Y. F. Qiu · Z. Y. Ma
Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Science, Shanghai 200241, People's Republic of China

Q. S. Zheng
Division of National Veterinary Biological Medicine Engineering Research Center, Nanjing, People's Republic of China

Keywords Bursal pentapeptide · Antiproliferation on tumor cell · Antitumor factor p53 · Hybridoma cell · Immunodominant functions

Introduction

The bursa of Fabricius (BF) was first implicated as the central humoral immune organ unique to birds with the discovery that surgical removal of the bursa from neonatal chicks impaired subsequent antibody responses to *Salmonella typhimurium* type O-antigen (Davison et al. 2008). It is reported that two distinct differentiation pathways for lymphocytes have been established. T (thymic) lymphocytes occur in the thymus, which is common in both chick and mammal. But, the B-cell-differentiating organ equivalent to the T-cell-differentiating thymus in mammal is in doubt. BF is critical for normal development of the B lymphocytes responsible for antibody production and Ig diversification (Davison et al. 2008; Ratcliffe 2006; Arakawa and Buerstedde 2004). Studies with BF extracts show that they contain multiple biological active factors. For example, Bursin selectively induces avian B cells from their precursor (Ann et al. 1976), and promotes immunoglobulin switching from IgM to IgG (Baba and Kita 1977). Except for anti-steroidogenic properties (Byrd et al. 1993), bursal anti-steroidogenic peptide (BASP) plays antiproliferative effects on mitogen-stimulated neonatal bursal-derived lymphocytes (Caldwell et al. 1999). Bursal septpeptide (BSP-II) enhanced the strong immune responses of mice and chickens immunized with avian influence virus (AIV) vaccine (Feng et al. 2010). Bursopentin (BP5) displayed strong immuno-stimulating activity by promoting B-cell proliferation directly and T-cell proliferation indirectly (Li et al. 2010).

Given the close relationship between BF and the B-cell differentiation and antibody production, it is important to study the bursal-derived biological peptides. In this study, we isolated a novel biological bursal peptide BPP-I from BF. The anti-proliferative role of BPP-I on MCF-7 cell was evaluated by MTT array. Furthermore, the potential functions of BPP-I on antitumor factor p53 transcriptional expression and protein expression were examined. We also examined the effects of BPP-I on antigen-specific immune response in immunized BALB/c mice with inactivated AIV (H_9N_2 strain) vaccine.

Materials and methods

Animals and cell lines

BALB/c female mice (6- to 8-week old, 17–21 g) were obtained from Yang Zhou University (Yangzhou, China). Studies were performed in accordance with the guidelines established by the Laboratory Animal Ethical Commission. Hybridoma cell producing IgG1 κ subtype MAbs with specificity to the Japanese encephalitis virus (JEV, SA14 strain), were grown and maintained in RPMI 1640 supplemented with 20% heat-inactivated fetal bovine serum.

Purification and sequence analysis of BPP-I

Bursal peptide was prepared from avian BF as previously described (Feng et al. 2010). In brief, BF tissue was homogenized in 0.85% NaCl (Dingguo, China). After centrifugation at 10,000*g* for 60 min, mixture was collected and dialyzed (lower than 1,000 Da) for 48 h at 4°C. The extract was filtered by 0.22 μm and was chromatographed through the 4.6 × 250 mm SinoChrom ODS-BP RP-HPLC affinity column (Elite, China) in a linear gradient of acetonitrile (2–100%) at a flow rate of 1 ml/min, with monitoring at 220 nm. The elution was collected and analyzed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (Bruker, Germany). Bursal peptide (BPP-I) was synthesised in Xi'an Huachen bio-technology Co., Ltd (China). The purity of synthetic peptide was over 97%.

The effect of the BPP-I to various cell lines

MCF-7, CEF, and PK15 were stimulated by BPP-I at different concentrations for 48 h. PBS was used as negative control. Cell viabilities were determined with the MTT (Sigma) (Avantika et al. 2010). The relative survival rate was calculated using the following equation: survival rate (%) = (absorbance of BPP-I-treated cells/absorbance of untreated control cells) × 100%.

The effect of the BPP-I to hybridoma cells

Hybridoma cells were stimulated by BPP-I at different concentrations for 48 h. PBS was used as negative control. Cell viabilities were determined with the MTT (Sigma) (Avantika et al. 2010). Antibody production from the hybridoma supernatant was determined as described previously (Feng et al. 2010).

Impact on p53 Luciferase activity and p53 protein expression

Luciferase assays in wild-type p53 Vero cells (Shen et al. 2009) were carried out in 24-well plates. Vero cells were transfected with p53 Luc and pRL-TK according to the manufacturer's instructions (Invitrogen). After 24 h, the transfected Vero cells were treated with BPP-I for 24 h. Luciferase activity was evaluated by dual-luciferase reporter system assay kit (Promega). Also, 20 μM α-pifithrin, inhibitor of p53 pathway (Walton et al. 2005), was added to transfected Vero cells for 2 h prior to the stimulation with BPP-I. Luciferase relative activity was assayed for 22 h later.

Vero cells were treated with BPP-I for 24 h. A positive control was carried out in parallel, treating cells with Dox (Sigma). Vero cells were lysed with cell culture lysis reagent (Promega). Western blotting was performed as previously described (Qiu et al. 2008) using anti-p53 monoclonal antibody (DO-1, Santa Cruz Biotechnology, USA), anti-β-actin monoclonal antibody (AC-15, Sigma, USA).

Mice model experiment

BALB/c mice were intraperitoneally immunized with BPP-I and inactivated AIV (H_9N_2 strain) vaccine. The prime-boost immunization procedure and dose per mouse are shown in Table 1. Three groups of mice were first

Table 1 The immunization diagram

Immunization group	Immunization sample		Dosage (per mouse) (mL)
	Vaccine	BPP-I concentration	
No. 1	PBS	–	0.2
No. 2	AIV vaccine	–	0.2
No. 3	AIV vaccine	10 μg/mL	0.2
No. 4	AIV vaccine	50 μg/mL	0.2
No. 5	AIV vaccine	250 μg/mL	0.2

AIV vaccine, inactivated avian influence virus (AIV, H_9N_2 strain) vaccine; PBS as negative control

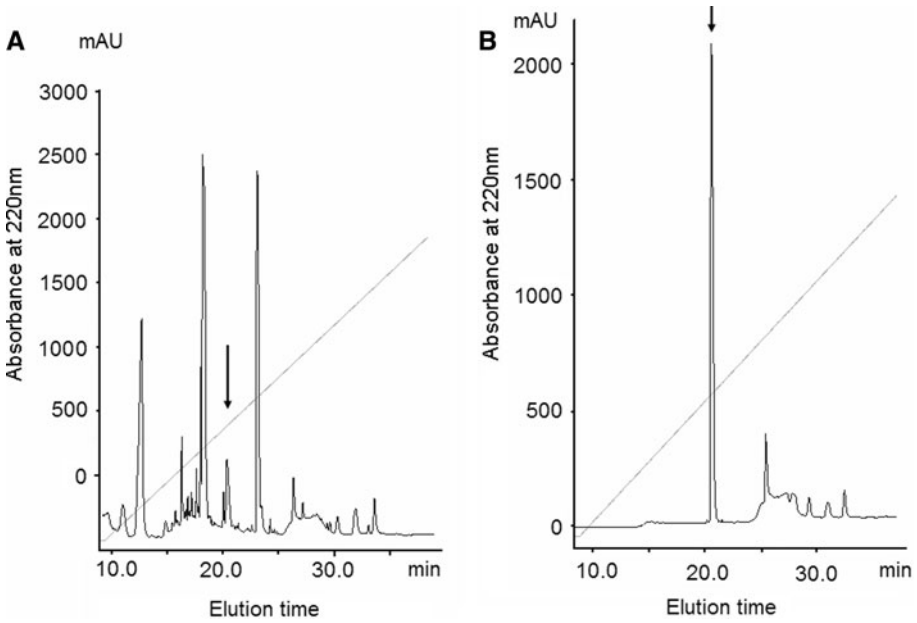

Fig. 1 Isolation and identification of BPP-I. **a** Purification of BPP-I. The extract was separated and purified on a 4.6 × 250 mm SinoChrom ODS-BP column with a linear acetonitrile gradient (*diagonal line*) by RP-HPLC. The retention peak time of BPP-I was 20.59 min (*the arrow pointed*). **b** Analytical RP-HPLC of purified BPP-I. The purity of BPP-I was assessed by RP-HPLC of BPP-I obtained from the peak (*arrow*) in (**a**) on a 4.6 × 250 mm SinoChrom ODS-BP column and developed with a linear (20–60%) acetonitrile gradient in 20.59 min

primed with AIV vaccine and BPP-I at 10, 50 and 250 μg/mL, respectively. Two weeks later, the mice were injected with AIV vaccine and three concentrations of BPP-I. Mice immunized with AIV vaccine only as a positive control, or 0.2 mL PBS as a negative control. All groups were immunized twice at 2-week intervals. Antibody subtype assay was conducted on serum collected at 14 days after the second immunization. The antibodies against AIV were analyzed by enzyme-linked immunosorbent assay (ELISA) (Feng et al. 2010), respectively. Also, the sera were collected on 1 week after the second immunization to measure the IL-4 and IFN-γ secretions by mice cytokine ELISA kits (RD, USA). Furthermore, the spleen cells were isolated from immunized mice as previously described method (Feng et al. 2010), and stimulated for 48 h with 10 μg/mL of Con A (positive control), 10 μg/mL of the AIV antigen (specific antigen stimulation), 5 μg/mL of BSA (irrelevant antigen), or no antigen (negative control), respectively, and the viability was measured using MTT-based method (Avantika et al. 2010). Also, immunophenotyping of spleen lymphocytes was performed by three-color flow cytometry analysis (BD, LSR), using different mixtures of specific mAbs labeled with PE, FITC or PE-Cy5.

Statistical analysis

Data obtained from different experiments were presented as mean ± SD from at least three independent experiments and evaluated by analysis of variance (ANOVA) or Student's *t*-test, using a significance level of 5%.

Results

Isolation and identification of natural BPP-I

Natural bursal peptide was isolated from avian BF by RP-HPLC method. In this paper, a new bursal pentapeptide I (BPP-I) was purified in elution peak time at 20.59 min (Fig. 1a), and the experimental sequence, LGPGP with the molecular weight 439.661 Da was identified by MALDI-TOF-MS. The purity of isolated BPP-I was assessed by RP-HPLC on a 4.6 × 250 mm SinoChrom ODS-BP column with a linear (20–60%) acetonitrile gradient (Fig. 1b). Aligning the BPP-I sequence by BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi), we found BPP-I shared identical amino acid sequence to numerous proteins of *G. gallus* species. From these analyses, we suggested that BPP-I is a five-amino-acid peptide processed from *G. gallus* species.

Potential roles in BPP-I-treated tumor cell and normal cells

In order to validate the probable anti-proliferative roles of BPP-I on tumor cell, we investigated BPP-I-treated MCF-7 cell viability. It is noteworthy that BPP-I reduced the MCF-7 cell viability in dose-dependent manners with a range 1–50 μg/mL (Fig. 2a). Relative viability rate in MCF-7 cell decreased by 24.88, 16.09, and 9% less for 50, 25 and 5 μg/mL BPP-I treatment, respectively (50 μg/mL, $P < 0.01$; 25 μg/mL, $P < 0.05$). However, BPP-I evoked an optimal proliferation response by 14.46% on MCF-7 at 0.1 μg/mL.

The effect of BPP-I on normal cell lines of CEF and Vero cell proliferation were tested with BPP-I (0.4–25 μg/mL).

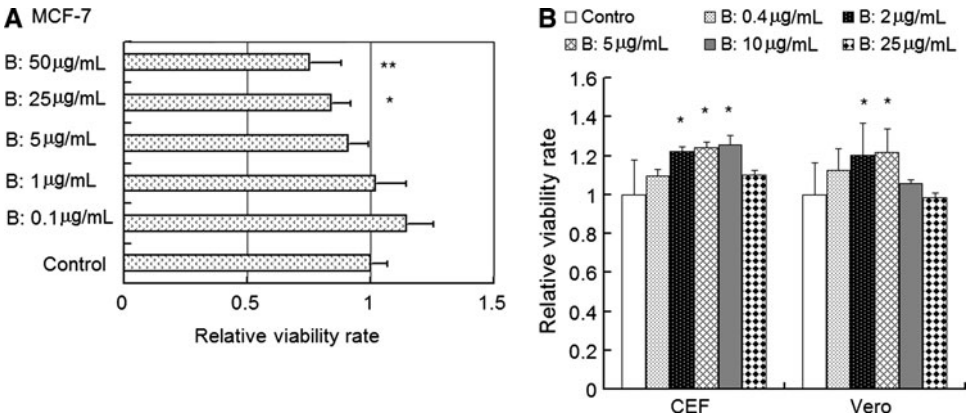

Fig. 2 BPP-I could play different roles on tumor cell and normal cell lines. **a** BPP-I decreased MCF-7 cell proliferation. MCF-7 cells (1×10^5 cells/mL) were treated with BPP-I for 48 h. MTT was added to analyze the viability. **b** BPP-I induced CEF and Vero cell proliferation. CEF and Vero cells were cultured and treated with BPP-I for 48 h, to detect both cells proliferation response. Data were expressed as mean ± SD of three experiments in duplicates. *B* BPP-I. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with PBS control without BPP-I treatment

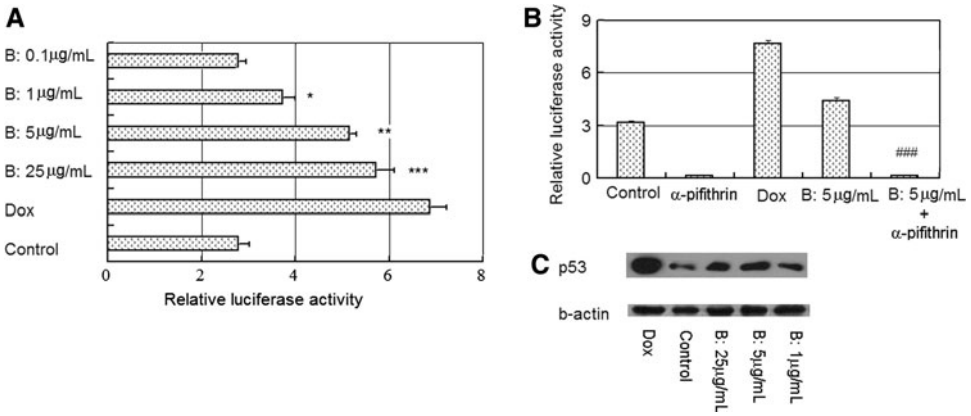

Fig. 3 BPP-I activated p53 expression. **a** BPP-I increased p53 luciferase activity. Vero cells were transfected and stimulated with BPP-I for 24 h. And p53 luciferase activity was determined. **b** α-pifithrin inhibited BPP-I-induced p53 luciferase activity. The transfected Vero cells were pro-incubated with α-pifithrin for 2 h, and stimulated with BPP-I at 5 μg/mL for 22 h to detect the p53 luciferase activity. **c** BPP-I activated p53 protein expression. Non-transfected Vero cells were stimulated with BPP-I for 24 h, and the p53 protein were determined by the west blot. Data were expressed as mean ± SD of three experiments in duplicates. Control without BPP-I treatment was used as a negative control, and Dox as a positive control. *B* BPP-I. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with PBS control without BPP-I treatment. $^{\#\#\#}P < 0.001$, compared with BPP-I (5 μg/mL)

The results showed that BPP-I (2–10 μg/mL) caused a significantly enhanced CEF cell proliferation indicated by MTT incorporation ($P < 0.05$) (Fig. 2b). Also, our experiments indicated that BPP-I could increase Vero cell proliferation. In comparison to control, BPP-I-treated Vero cell proliferation was increased by about 21% at 2 and 5 μg/mL (Fig. 2b). No significant inhibitions on CEF and Vero cell proliferation were observed with BPP-I treatment.

Role on p53 luciferase activity and p53 protein expression

To further understand the role of p53, we investigated the p53 transcriptional function during the BPP-I treatment. To achieve this, we transfected p53 Luc and pRL-TK into Vero cells that were BPP-I-treated 24 h after transfection. Luciferase activity was significantly enhanced during the BPP-I treatment from 25 to 1 μg/ml (25 μg/mL, $P < 0.001$; 5 μg/mL, $P < 0.01$; 1 μg/mL, $P < 0.05$) (Fig. 3a), indicating that BPP-I is able to induce the transcriptional activation of p53 in a dose-dependent manner. In contrast, luciferase levels were not observed in non-transfected Vero cells after BPP-I treatment (data not shown). To inhibit p53 transcriptional activation, transfected Vero cells were treated with a specific p53 inhibitor, 20μM α-pifithrin (Walton et al. 2005), for 2 h before BPP-I treatment. The results showed that α-pifithrin significantly inhibited BPP-I-induced p53 luciferase activity ($P < 0.001$) (Fig. 3b).

Increased p53 levels do not necessarily correlate with increased transcriptional activity (Kim and Deppert 2003).

To determine whether the upregulation of p53 was induced by BPP-I treatment, we examined p53 protein levels in Vero cells treated with BPP-I with different concentrations that activated p53 transcriptional activity. After exposure to BPP-I, an increase in the level of the p53 was detected in Vero cell (Fig. 3c) in a dose-dependent manner.

Taken together, these findings indicated that BPP-I-induced p53 protein expression level was correlated with increased transcriptional activity.

BPP-I-induced antibody production of hybridoma cell

In this paper, we chose the hybridoma SP2/0 as an immune cell model in vitro which possesses the characteristics of B lymphocyte secreting antibody. Hybridoma cells were stimulated with BPP-I for 48 h. As shown in Fig. 4a, hybridoma cell treated with BPP-I produced significantly higher anti-JEV antibody (IgG1) titers (0.01 μg/mL, $P < 0.05$; 0.1 and 5 μg/mL, $P < 0.01$; 1 μg/mL, $P < 0.001$) than those PBS control without treatment with BPP-I. Also, BPP-I could enhance hybridoma cell viability increase at 0.1 μg/mL ($P < 0.05$) (Fig. 4b).

Potential functions on immune response in mice

As biological peptide from avian humoral immune system, we first investigated the role of BPP-I on spleen lymphocyte proliferation. The results showed that the cell proliferation of spleen lymphocyte from the immunized mice with AIV vaccine and BPP-I was significantly increased (10 and 250 μg/mL, $P < 0.05$; 50 μg/mL, $P < 0.01$), compared with that of AIV vaccine control, and the inducing activity on lymphocyte proliferation is related to dose of BPP-I, in which the highest proliferation response was presented in the immunization group with AIV vaccine and 50 μg/mL BPP-I among all immunization experiment groups (Fig. 5a). To estimate the potential functions on the humoral immune response, the antibody and subtype in sera from immunized mice were measured by ELISA. Knowing that BF is responsible for the antibody production, in this paper, we began with assaying the titers of serum IgG from each individual immunized mouse at 2 weeks after the second immunization (Fig. 5b, IgG). All mice immunized with BPP-I generated a significant AIV-specific IgG antibody response (10 and 250 μg/mL, $P < 0.05$; 50 μg/mL, $P < 0.01$), and mice receiving 50 μg/mL BPP-I immunizations generated the highest antibody response among all the experiment groups which implied that BPP-I might have the noticeable adjuvant effect on the immunized mice.

To establish the antibody subtypes, we determined the IgG2a and IgG1 antibody subtypes collected from each group (Feng et al. 2010). The results showed that IgG1 subtype was dominant in all immunized mice, and the lower amount of IgG2a subtype was detected in the sera from immunized mice (Fig. 5b, IgG1 and IgG2a). The results suggest that IgG1 subtypes may play a major role in the immunized mice with AIV vaccine and BPP-I.

The cytokines play an important role in T-cell differentiation, cell-mediated and humoral immunity. In this paper, we also explored the characterization of the IL-4 and IFN-γ cytokines in sera collected from immunized mice at seventh day after the second immunization. As shown in Fig. 5c, IL-4 and IFN-γ productions were significantly

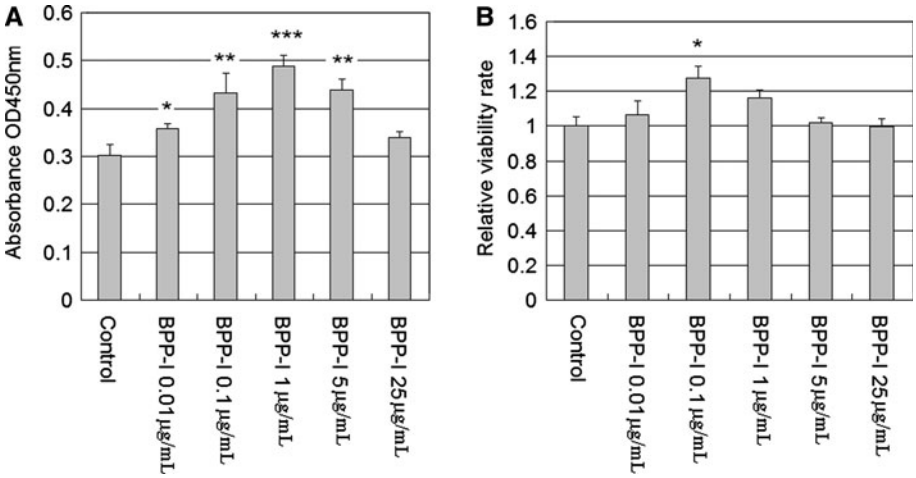

Fig. 4 Effect of BPP-I on hybridoma cell. **a** Effects of BPP-I on antibody production of hybridoma cell. Hybridoma (1×10^5 cells/mL) was stimulated with BPP-I for 48 h. The levels of antibody were determined by ELISA. **b** Effects of BPP-I on hybridoma proliferation. Hybridoma viabilities were measured by MTT assay at absorbance 570 nm. *B* BPP-I. Data are shown as mean ± SD for a representative experiment from three experiments, each performed in triplicate. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with PBS control without BPP-I treatment

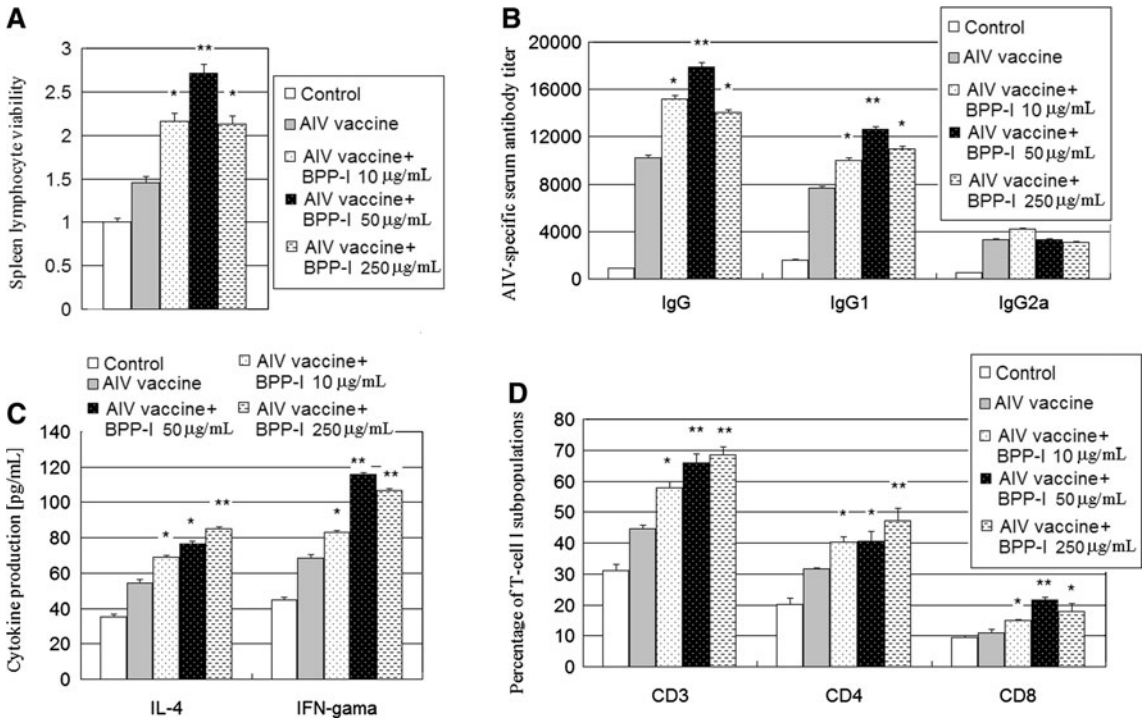

Fig. 5 BPP-I induced strong immune response on mice experiments. BALB/c mice were twice immunized with BPP-I and AIV H9N2 strain inactivated vaccine. **a** Co-treatment of BPP-I and inactivated AIV vaccine stimulated virus-specific spleen lymphocyte proliferation responses. Splenic lymphocytes from immunized mice on day seven after second immunization were prepared for detecting lymphocyte viability by MTT. **b** BPP-I stimulated significant antigen-specific humoral mediated immune response. Sera were collected on day 28 for detecting the antibody (IgG) and subtypes (IgG1 and IgG2a) titers special to AIV by ELISA. **c** Effect of different doses of BPP-I and inactivated AIV vaccine on Th1/Th2 cytokine productions. On the seventh day after the second immunization, sera were collected to measure Cytokine by using sandwich ELISA method using ELISA kits. **d** Analysis of lymphocyte subsets from spleen of immunized mice. T-cell sub-types percentage by monitoring fluorochrome conjugated mAbs of the corresponding PE/FITC/PE-Cy5 conjugated mAbs by flow cytometry. Data represent the mean ± SD of three independent observations performed in triplicate. $*P < 0.05$, $**P < 0.01$, compared with mice immunized with the inactivated AIV vaccine alone

increased in a dose-dependent manner in BPP-I co-immunized groups (IL-4: 10 and 50 μg/mL, $P < 0.05$; 250 μg/mL, $P < 0.01$; IFN-γ: 10 μg/mL, $P < 0.05$; 50 and 250 μg/mL, $P < 0.01$). The IL-4 and IFN-γ were the representative cellular factor of Th2-type and Th1-type immune response, respectively.

Moreover, populations of splenic lymphocyte immunophenotypic T cells induced by inactivated AIV were also elevated by BPP-I (Fig. 5d). The percentages of CD3+, CD3+CD4+ cells and of CD3+CD8+ cells, were significantly higher in the BPP-I co-immunization groups than that of AIV vaccine control (CD3: 10 μg/mL, $P < 0.05$; 50 and 250 μg/mL, $P < 0.01$; CD4: 10 and 50 μg/mL, $P < 0.05$; 250 μg/mL, $P < 0.01$; CD8: 10 and 250 μg/mL, $P < 0.05$; 50 μg/mL, $P < 0.01$).

Discussion

Immunology is not just restricted to investigations on the immune systems of mice and humans. Immunology studies on other vertebrate species have contributed greatly to the development of immunological understanding. Among these species, bird immune system has provided an invaluable model for investigating basic immunological mechanisms. BF is a vital lymphoid organ in the immune system unique to birds, and has a primary role in the B-lymphocytes development and function (Davison et al. 2008), after which immune lymphocyte B cell is named. In this study, a new bursal pentapeptide I (BPP-I), LGPGP, was isolated from avian humoral immune system (Fig. 1), and shared the identical or similar amino acid sequence to various proteins of *G. gallus* species. Among these homologous proteins by BLAST analysis, CAH65368 is from the lymphocyte and the bursa of avian at a 2-week-old stage (Caldwell et al. 2005). One region of CAH65368, RCC1, first reported as the regulator of chromosome condensation 1 (Ohtsubo et al. 1987), is the guanine nucleotide exchange factor for Ran GTPase, which regulates critical eukaryotic cellular functions including nuclear transport (Makde et al. 2010). To our knowledge, this is the first time to report the bursal pentapeptide with LGPGP.

As a bursal-derived bioactive peptide, the roles of BPP-I were never reported. In this paper, we first reported the biological multifunction of BPP-I. It was reported that breast cancer is the most common cancer among women with high female cancer lethality. MCF-7 human breast adenocarcinoma cell line is the common experimental cell line of breast cancer origin (Lacroix and Leclercq 2004). In this paper, we used MCF-7 as a tumor cell model in vitro to study the potential function of BPP-I on cancer cell proliferation (Fig. 2a). It was found that BPP-I could inhibit tumor cell MCF-7 proliferation at reachable concentrations range (from 1 to 50 μg/mL). However, no inhibitions on normal cell proliferation of CEF and Vero cell were observed with BPP-I treatment. Therefore, we thought that BPP-I might specially decrease tumor cell proliferation. Moreover, BPP-I triggered antitumor factor p53 luciferase activity and p53 protein expression (Fig. 3), which is well known to play a crucial role in inducing apoptosis and acting as cell cycle checkpoints in human and murine cells following DNA damage (May and May 1999). There were three principal mechanisms including tumor growth, defective immunorecognition and immunosuppression, which were considered responsible for immune evasion (Nicolini and Carpi 2009). Thus, the immune system might be a major target strategy to develop novel antitumor therapies (Gajewski et al. 2009). It was proved that B cells could play a role in antitumor cellular responses against a lung metastatic tumor and in the removal of B cells from lung lymphocyte cultures resulted in diminished IFN-γ secretion and tumor lysis, which provide evidences of the importance of B cell responses in tumor defenses (Jones et al. 2008). BF is a vital immune organ responsible for B cell difference (Davison et al. 2008). Therefore, we thought that these results are important in the context that the humoral immunity against tumors could be enhanced by BPP-I, a bioactive peptide from avian humoral system.

BF is the central humoral immune organ unique to avian, and plays vital roles on antibody production (Davison et al. 2008). The results of hybridoma immune cell model which shares the characteristics of B lymphocyte secreting antibody proved that BPP-I-induced antibody production of hybridoma cell in a dose-dependent manner (Fig. 4), which suggested that BPP-I was a novel bursal-derived immune inducing factor for antibody production of hybridoma cell line.

Due to the biological active peptides isolated from BF, we hypothesized that BPP-I would play role on immune responses. This prediction was confirmed in mice immunized with AIV vaccine and BPP-I which generated a strong antibody production increase, with IgG1 antibody subtype exclusively (Fig. 5b). It was reported that IgG1 was taken as an indicator of Th2-type immune response (Bungener et al. 2008). These results indicated that the AIV vaccine-BPP-I was capable of enhancing the immune response toward a Th2-type immune response.

Some small molecular peptides were reported to enhance cell-mediated immune response. Thymopentin (TP5), derived from the thymus, stimulates T-cell differentiation and enhances cellular immune response (Singh et al. 1998). We wondered whether BPP-I might play roles on cell-mediated immune response. The results verified that BPP-I augmented the level of Th1-type and Th2-type cytokines (Fig. 5c). Cytokines induce multifarious biological effects on different cell types in immune processes. IL-4 promotes the differentiation of naive CD4+ T cells into Th2 cells (Seder et al. 1992), and IFN-γ enhances both CD8+ T-cell-mediated and non-specific cell-mediated immune responses (Bou Ghanem et al. 2011). Also, in this paper, BPP-I enhanced T-cell immunophenotyping of the spleen lymphocyte from immunized mice (Fig. 5d). Therefore, we thought that BPP-I, derived from the central humoral immune organ unique to birds, might play immune inducible roles not only on humoral immune response but also on cell-mediated immune response resulting in a balanced immune system of organism. These results suggested that BPP-I acted as a multi-functional modulator in immunized mice.

Due to birds and mammals evolving from a common reptilian ancestor more than 200 million years ago and having inherited many common immunological systems, the avian immune system provides an invaluable model for studies on basic immunology (Davison et al. 2008). So, it is valuable to study the immunodominant roles of new bursal peptide from this central humoral immune organ. In this paper, we demonstrated that a novel bursal-derived pentapeptide from avian humoral immune system presents potential role on antitumor-cell-proliferation and activating antitumor protein p53 expression. Also, BPP-I plays powerful immunomodulatory functions on humoral and cell-mediated immune responses in animal immunization experiments. This study indicates that BPP-I might act as a probable candidate reagent for antitumor and immune improvement uses.

Acknowledgements This work was supported by the National Agriculture Special Research Project for Non-Profit Trades, Ministry of Agriculture (No. 200803020). Also, we thank Elsevier Language Editing Services (https://languageediting.elsevier.com/) for professional language editing service in the preparation of this manuscript.

Conflict of interest The authors declared no conflict of interest.

References

Ann B, Douglas GG, Gideon G (1976) Lymphocyte-differentiating hormone of bursa of Fabricius. Science 193:319–321

Arakawa H, Buerstedde JM (2004) Immunoglobulin gene conversion: insights from bursal B cells and the DT40 cell line. Dev Dyn 229:458–464

Avantika V, Kashi NP, Aloukick KS, Kishan KN, Rakesh KG, Vimal KP (2010) Evaluation of the MTT lymphocyte proliferation assay for the diagnosis of neurocysticercosis. J Microbiol Methods 81(2):175–178

Baba T, Kita M (1977) Effect of extracts of the bursa of Fabricius on IgG antibody production in hormonally bursectomized chickens. Immunology 32:271–274

Bou Ghanem EN, Nelson CC, D'Orazio SE (2011) T cell-intrinsic factors contribute to the differential ability of CD8+ T cells to rapidly secrete IFN-γ in the absence of antigen. J Immunol 186(3):1703–1712

Bungener L, Geeraedts F, Ter Veer W, Medema J, Wilschut J, Huckriede A (2008) Alum boosts TH2-type antibody responses to whole-inactivated virus influenza vaccine in mice but does not confer superior protection. Vaccine 26(19):2350–2359

Byrd JA, Dean CE, Hayes TK, Wright MS, Hargis BM (1993) Detection and partial characterization of an anti-steroidogenic peptide from the humoral immune system of the chicken. Life Sci 52:1195–1207

Caldwell DJ, Dean CE, McElroy AP, Caldwell DY, Manning JG, Hargis BM (1999) Bursal anti-steroidogenic peptide (BASP): modulation of mitogen-stimulated bursallymphocyte DNA synthesis. Comp Biochem Physiol A Mol Integr Physiol 123(4):385–391

Caldwell RB, Kierzek AM, Arakawa H, Bezzubov Y, Zaim J, Fiedler P, Kutter S, Blagodatski A, Kostovska D, Koter M, Plachy J, Carninci P, Hayashizaki Y, Buerstedde JM (2005) Full-length cDNAs from chicken bursal lymphocytes to facilitate gene function analysis. Genome Biol 6(1):R6

Davison F, Kaspers B, Schat KA (eds) (2008) Avian Immunology, Elsevier/Academic Press, New York

Feng XL, Su XD, Wang FQ, Wei JC, Wang FJ, Cao RB, Zhou B, Mao X, Zheng QS, Chen PY (2010) Isolation and potential immunological characterization of TPSGLVY, a novel bursal septpeptide isolated from the bursa of Fabricius. Peptides 31:1562–1568

Gajewski TF, Chesney J, Curriel TJ (2009) Emerging strategies in regulatory T-cell immunotherapies. Clin Adv Hematol Oncol 7(1):1–10 (quiz 11–12)

Jones HP, Wang YC, Aldridge B, Weiss JM (2008) Lung and splenic B cells facilitate diverse effects on in vitro measures of antitumor immune responses. Cancer Immun 8:4

Kim E, Deppert W (2003) The complex interactions of p53 with target DNA: we learn as we go. Biochem Cell Biol 81(3):141–150

Lacroix M, Leclercq G (2004) Relevance of breast cancer cell lines as models for breast tumours: an update. Breast Cancer Res Treat 83(3):249–289

Li DY, Geng ZR, Zhu HF, Wang C, Miao DN, Chen PY (2010) Immunomodulatory activities of a new pentapeptide (Bursopentin) from the chicken bursa of Fabricius. Amino Acids. doi: 10.1007/s00726-010-0663-7

Makde RD, England JR, Yennawar HP, Tan S (2010) Structure of RCC1 chromatin factor bound to the nucleosome core particle. Nature 467(7315):562–566

May P, May E (1999) Twenty years of p53 research: structural and functional aspects of the p53 protein. Oncogene 18(53):7621–7636

Nicolini A, Carpi A (2009) Immune manipulation of advanced breast cancer: an interpretative model of the relationship between immune system and tumor cell biology. Med Res Rev 29(3):436–471

Ohtsubo M, Kai R, Furuno N, Sekiguchi T, Sekiguchi M, Hayashida H, Kuma K, Miyata T, Fukushige S, Murotsu T et al (1987) Isolation and characterization of the active cDNA of the human cell cycle gene (RCC1) involved in the regulation of onset of chromosome condensation. Genes Dev 1(6):585–593

Qiu Y, Shen Y, Li X, Liu Q, Ma Z (2008) Polyclonal antibody to porcine p53 protein: a new tool for studying the p53 pathway in a porcine model. Biochem Biophys Res Commun 377(1):151–155

Ratcliffe MJH (2006) Antibodies, immunoglobulin genes and the bursa of Fabricius in chicken B cell development. Dev Comp Immunol 30:101–118

Seder RA, Paul WE, Davis MM, Fazekas de St Groth B (1992) The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of CD4 + T cells from T cell receptor transgenic mice. J Exp Med 176(4):1091–1098

Shen Y, Wang X, Guo L, Qiu Y, Li X, Yu H, Xiang H, Tong G, Ma Z (2009) Influenza A virus induces p53 accumulation in a biphasic pattern. Biochem Biophys Res Commun 382(2):331–335

Singh VK, Biswas S, Mathur KB, Haq W, Garg SK, Agarwal SS (1998) Thymopentin and splenopentin as immunomodulators. Immunol Res 17:345–368

Walton MI, Wilson SC, Hardcastle IR, Mirza AR, Workman P (2005) An evaluation of the ability of pifithrin-alpha and -beta to inhibit p53 function in two wild type p53 human tumor cell lines. Mol Cancer Ther 4(9):1369–1377