Mahidol University Journal of Pharmaceutical Sciences 2007; 34(1-4): 53-59.

Original Article

Effect of OVS1 Monoclonal Antibody as Targeted Ovarian and Breast Cancer Therapy

P. Moongkarndi,[1*] N. Jaisupa,[1] S. Jongsomboonkusol,[1]
S. Kaslungka,[2] N. Sengpanich,[1] W. Samosorn,[1]
N. Kosem[1] and N. Neungton[3]

[1]*Faculty of Pharmacy, Mahidol University,*
[2]*The Government Pharmaceutical Organization,* [3]*Faculty of Medicine,*
Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Abstract Cancer is ranked among the top three causes of death in Thailand. The research has been focused to improve the diagnosis and treatment of cancers. Monoclonal antibodies could become one of the major tools to achieve the satisfied outcome. OVS1 monoclonal antibody (MAb) produced against ovarian cancer was used to identify the mucinous cystadenocarcinoma antigen as a tumor marker secreted in serum. Since breast and ovarian cancers shared various associate markers, the potential of OVS1 MAb in ovarian and breast cancer treatments was determined. MTT assay was employed to measure the cytotoxicity against breast (BT549), ovarian (SKOV3) and endothelial (ECV304) cell lines. Paclitaxel, an anti-tumor drug, and α-mangostin, a natural purified constituent from *Garcinia mangostana* pericarp, were tested as MAb-drug conjugation. The binding efficiency of OVS1-α-mangostin to the cancer cells was monitored by fluorescein-5-isothiocyanate (FITC). Furthermore, the apoptosis of cancer cell lines was examined using fluorimetric assay and confirmed by DNA fragmentation in gel electrophoresis. The ED_{50} of α-mangostin were 3.5 and 3.4 μg/ml, while the ED_{50} of MAb-α-mangostin conjugation (MAb-drug conjugation) were 29.6 and 42.8 μg/ml against SKOV3 and ECV304, respectively. Although the ED_{50} of MAb-drug (α-mangostin) conjugation was higher than that of α-mangostin alone, the binding efficiency and specificity of MAb-α-mangostin to SKOV3 cancer cells were satisfactory as monitored using FITC. Furthermore, the ED_{50} of MAb-α-mangostin conjugation against SKOV3 cancer cell showed significant lower value than that against ECV304 endothelial cell line. This study supported the potential application of MAb-drug or -medicinal plant conjugation as anti-cancer drug for targeted cancer therapy.

Keywords: MAb-drug conjugation, α-mangostin, OVS1 MAb, paclitaxel, targeted cancer therapy

INTRODUCTION

There were approximately 24.6 million people living with cancer in year 2006 and almost 7 million people die each year. By 2020, patients with cancer will be estimated at 30 million, whereas mortality rate will be over 10 million a year. Moreover, the estimated number of new cases annually is expected to rise from 10 million in 2002 to 16 million by 2020 if this trend continues.[1,2] Cancer is ranked among the top three causes of death in Thailand.[3] Breast cancer is in the first rank of common cancers in females. However, ovarian cancer is the leading cause of death from gynecologic malignancies, since the tumor is often in an advanced stage by the time of clinical diagnosis. Although some progress has been made in cancer diagnosis and treatment, the high incidence and low survival rate of patient have still been reported. The development of new therapeutic approach remains one of the most challenging in cancer research.[4-7]

[*]**Corresponding author:** Faculty of Pharmacy, Mahidol University, 447 Sri-Ayudhaya Road, Bangkok 10400, Thailand. Fax: (662) 644-8692. Email: pypmk@mahidol.ac.th

Targeted therapies involve production of components especially monoclonal antibodies may best be used in the short term, combination with traditional therapies.[8,9] More research is needed to identify which cancers may be best treated with targeted therapies. Therapies that target receptors are mostly applied with monoclonal antibodies.

OVS1 monoclonal antibody (MAb) produced against fresh human ovarian cystadenocarcinoma cells, was selected by immunohistochemistry and showed 96% specificity and 67% sensitivity to mucinous cystadenocarcinoma without cross reaction to normal cells, *i.e.,* benign or non-ovarian cancer tissues.[10] The result confirmed the highly specific and moderately sensitive recognition of OVS1 antigen presented on cancer tissue. OVS1 MAb could determine the specific antigen levels in serum and evaluate them as tumor markers. It has been reported to be beneficial to cancer patients.[4]

Apart from the benefit of OVS1 MAb for diagnosis, the application of OVS1 MAb for cancer treatment was studied. Cytotoxic and apoptotic activities of OVS1 MAb against ovarian and breast cancer were investigated. OVS1 MAb and paclitaxel (beneficial for advanced ovarian cancer patients who have become resistant to standard chemotherapy) convincingly induce apoptosis on SKOV3 and BT549 cells studied by two-color fluorescent DNA staining and agarose gel electrophoresis.[9] Combined application of OVS1 MAb and paclitaxel can enhance the cytotoxic activity compared with either OVS1 MAb or paclitaxel given alone on both cell lines. This suggestion for the enhanced cytotoxic activity may be due to the different mechanism of action of these two substances.

Many potential cancer-protective agents, especially from phytochemicals can be categorized as blocking agents, which impede the initiation stage, or suppressing agents, which arrest or reverse the promotion and progression of cancer, presumably by affecting or perturbing crucial factors that control cell proliferation, differentiation, senescence or apoptosis. The pericarp of the *Garcinia mangostana* has been used as a folk medicine for many years to treatment of skin infection, wound and dysentery diarrhea. It has been revealed to have major biological active compounds, α- and γ-mangostin, and other minor xanthones.[11] Among these compounds, α-mangostin is one of the interesting constituent, which generally found in the various parts of this plant especially in the pericarp, and has been reported to possess interesting pharmacological activities, such as anti-vancomycin resistant enterococci, induction apoptosis of human leukemia,[12] inhibition the oxidation modification of human LDL, histamine H_1 receptor antagonist, anti-MRSA and effect on Ca^{2+}-ATPase. In this study, we conjugated OVS1 MAb with our purified α-mangostin and labeled with FITC, then observed the activity of the MAb-Drug conjugate in SKOV3 (ovarian cancer) and ECV304 (endothelial) cell lines.

MATERIALS AND METHODS

Monoclonal Antibody Purification

OVS1 MAb was produced and maintained according to Neungton, *et al.*[10] The supernatant from OVS1 hybridoma cell culture medium (RPMI 1640 supplemented with 10% fetal calf serum and other additives) was collected and applied to the Protein A-Sepharose column for the antibody purification.

MTT Assay

MTT assay was used to assess antiproliferative activity of extract on cancer cells. The survival cells after exposure could reduce the yellow MTT tetrazolium salt to insoluble purple formazan crystal by succinate dehydrogenase.[13] SKOV3 cells (1.5×10^4 cells/100 μl) were seeded into each well and incubated for 24 hours. Medium was removed and replaced with extract and incubated for indicated time. After exposure, medium was discarded and incubated with MTT solution (1 mg/ml, 50 μl/well) for 1 hour. Isopropanol was used to dissolve formazan crystal and plates were gently shaken for 5 minutes. Survival cells were proportional to the intensity of purple formazan determined by microplate reader at 590 nm ($A_{590\ nm}$ = absorbance at 590 nm).

Survival cells (%) = ($A_{590\ nm}$ of treated cells / $A_{590\ nm}$ untreated cells) x 100

Ladders of DNA Fragmentation

The presence of oligonucleosome-sized fragmentation of DNA which produce ladders, can demonstrate apoptotic cell death after exposure to the extract.[14] SKOV3 cells (1.5 x 10^6 cells/10 ml) were seeded into each dish and incubated for 24 hours. Cells were exposed with extract for indicated time. After exposure, cells were detached by gently scraping and packed to cell pellets by centrifugation at 5,000 r.p.m. for 5 minutes. Cells were washed with PBS once, lysis buffer was added in each tube and then incubated at 50°C for 1 hour. Proteinase K (20 mg/ml, 10 µl) was then mixed and continuously incubated at 50°C for 30 minutes. Cell suspensions were then added with RNase A (10 mg/ml, 3 µl) and incubated at 50°C for 2 hours. To remove proteins and other wastes from DNA solution, chloroform:isoamyl alcohol (24:1) were added into each tube and centrifuged at 14,000 r.p.m. for 10 minutes. DNA fragments dissolved in upper supernatant could be detected using 1.5% agarose gel electrophoresis.

Purification of α-Mangostin

Based on the results from the preliminary activities and TLC analysis of mangosteen extract, EtOAc extract was chosen to purify α-mangostin as a major active constituent. EtOAc extract was chromatographed on a silica gel-column chromatography using gradient solvent system in various proportions of hexane, EtOAc and MeOH as mobile phase. The isolated fractions consisted of α-mangostin could be visualized using TLC analysis. Each fraction with the similar pattern of TLC chromatogram was pooled and used for further purification. High-performance liquid chromatography (HPLC), infrared (IR) spectroscopy, mass spectrometry (MS) and nuclear magnetic resonance (NMR) analysis were performed to confirm the purity and elucidate the structure of α-mangostin.

MAb Conjugation and Activity Iidentification

In order to determine the potential of OVS1 MAb in ovarian cancer and breast cancer treatments, MTT assay was employed to measure the cytotoxicity against SKOV3, BT549 and ECV304 cell lines. Paclitaxel, an anti-tumor drug, and α-mangostin, a natural purified constituent from *Garcinia mangostana* pericarp, were employed as drug control and as MAb-drug conjugation. The binding efficiency of MAb-α-mangostin conjugation to the cancer cells was monitored by fluorescein-5-isothiocyanate (FITC).[15-17] Furthermore, the apoptosis of both cancer cell lines was examined using fluorimetric assay and confirmed by DNA fragmentation in gel electrophoresis.

RESULTS AND DISCUSSION

Many new discovered anticancer drugs are derived from natural products and biological substances. Most of the discovery based on their pharmacological properties in traditional medicine wisdom. Although novel technologies are applied in health care and medical process, anticancer drug discovery is still in need, since the highly effective drug with less toxicity is one of the crucial fields for the treatment of cancer.

Mangosteen is one of the famous fruits and has been widely used in traditional medicine. Our previous study found that crude extract from mangosteen possessed the cytotoxic and apoptotic activities and has high potential role for searching as anticancer drug.[18] α-Mangostin was a major purified constituent isolated from this work and reported to have high cytotoxic and apoptotic activities.[19] Combination of monoclonal antibody and drug or natural substance would be a new trend of treatment as targeted therapy to fight against cancer. Therefore, our established OVS1 monoclonal antibody against ovarian cancer and α-mangostin isolated from our local Thai fruit pericarp were conjugated and studied their activities. The OVS1 MAb was successfully purified by Protein A affinity chromatography as demonstrated in Figure 1 and Figure 2. The MAb also showed the apoptotic activity by DNA fragmentation

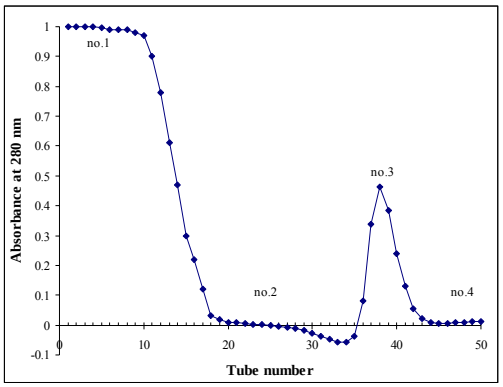


Figure 1. Purification of OVS1 MAb by Protein A Sepharose 4B affinity chromatography.

no.1 OVS1 MAb supernatant was loaded into column.

no.2 Unbound proteins were removed through column.

no.3 OVS1 MAb was eluted by 0.1 M sodium citrate buffer pH 3.0.

no.4 Column was washed after elution and equilibrated with 0.05 M PBS pH 7.4.

analysis (Figure 3). α-Mangostin was also obtained as pure constituent isolated from magosteen pericarp. The structure was demonstrated in Figure 4.

The ED_{50} of OVS1 MAb against SKOV3 and BT549 cell lines were 26.25 and 25.00 µg/ml and those of paclitaxel were 21.88 and 9.20 nM, respectively. The quantitative amount of cells determined by fluorimetric assay was correlated to the results of the MTT assay. The combination of OVS1 MAb and paclitaxel (MAb-drug combination) applying on these two cell lines resulted in a greater cytotoxicity than observed by either agent alone. The rate of apoptosis was also enhanced by the combination of these two substances. DNA fragmentation was detected in an agarose gel electrophoresis after treating cells with OVS1 MAb and paclitaxel at 24 hours (Figure 3).

In addition, the ED_{50} of α-mangostin against SKOV3 and ECV304 were 3.5 and 3.4 µg/ml, while those of OVS1 conjugated with α-mangostin (MAb-drug conjugation) were 29.6 and 42.8 µg/ml, respectively.

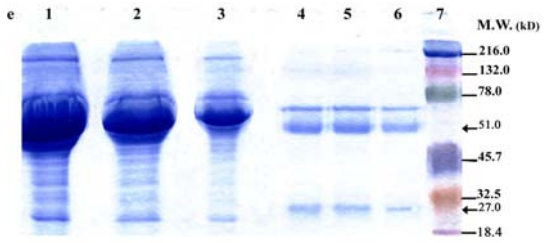


Figure 2. Polyacrylamide gel electrophoresis of OVS1 monoclonal antibody. Slab gel contained 7.5% and 4.0% acrylamide gel of separating and stacking gels, respectively.

Lane 1-3: OVS1 MAb supernatant before purification.

Lane 4-6: OVS1 MAb supernatant after purification.

Lane 7: Kaleidoscope protein standard marker.

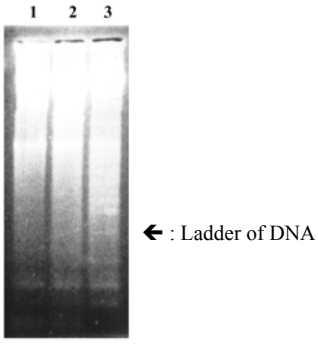


Figure 3. Analysis of DNA fragmentation in 1.5% agarose gel electrophoresis after treating SKOV3 cells with OVS1 MAb and paclitaxel.

Lane 1: SKOV3 control cells.

Lane 2: Cells were treated with 25 nM paclitaxel at 24 hours.

Lane 3: Cells were treated with 25 µg/ml OVS1 MAb at 24 hours.

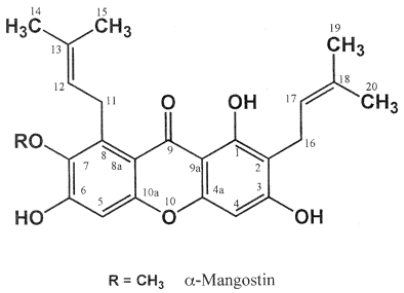


Figure 4. Structure of α-mangostin.

Although the ED_{50} of MAb-α-mangostin conjugation was higher than that of α-mangostin alone (Figure 5), the binding efficiency and specificity of MAb-α-mangostin to the cancer cells were satisfactory as monitored using FITC. Furthermore, the ED_{50} of MAb-α-mangostin conjugation against SKOV3 ovarian cancer cell lines showed significant lower value than ECV304 endothelial cell line. This result demonstrated a high potential of MAb-α-mangostin utilization as an anti-cancer drug for targeted cancer therapy (Figures 6 and 7).

CONCLUSION

These findings on the induction of cytotoxicity and apoptosis by OVS1 MAb on cancer cell lines and MAb-drug conjugation implied that OVS1 MAb has a high potential for the application in clinical therapy. MAb-α-mangostin conjugation showed more specificity on tumor cells than α-mangostin alone. With further *in vitro* and *in vivo* experiments, this could be summarized and carried on study as a novel drug for ovarian and breast cancer treatments.

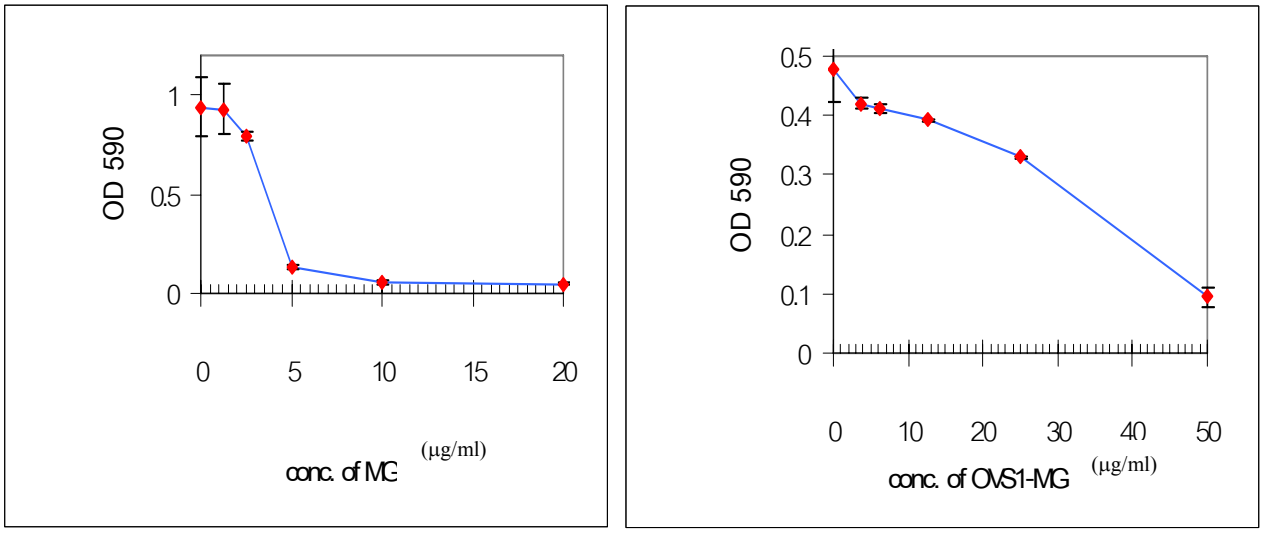


Figure 5. MTT cytotoxicity assay of α-mangostin (MG) and OVS1-α-mangostin (OVS1-MG) against SKOV3. The ED_{50} of MG and OVS1-MG on SKOV3 were 3.4 and 29.6 µg/ml, respectively.

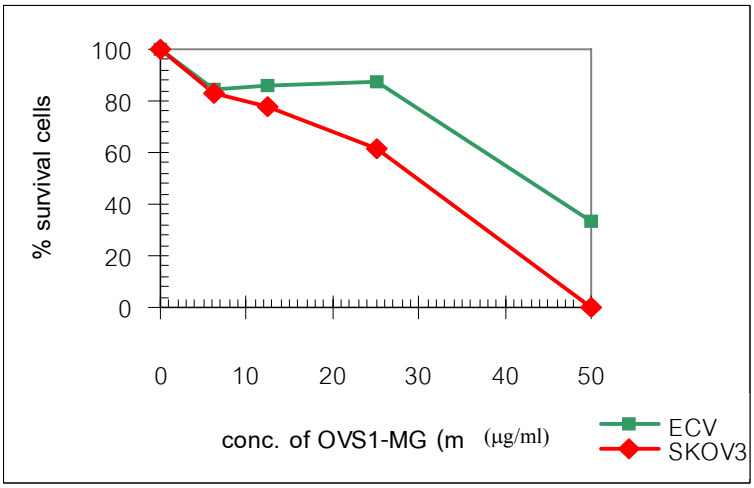
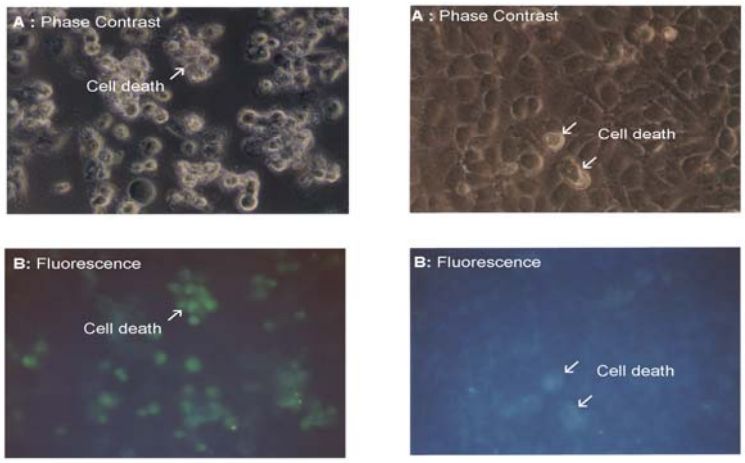


Figure 6. Comparison of MTT cytotoxicity assay of OVS1-α-mangostin (OVS1-MG) against SKOV3 and ECV304 cells. The graph demonstrates that OVS1-MG reacted more specific with ovarian tumor cell (SKOV3) than endothelial cells (ECV304).



(a) (b)

Figure 7. Binding of OVS1-α-mangostin-FITC with SKOV3 ovarian cancer cells (a) could be clearly demonstrated comparing with ECV304 endothelial cells (b).

ACKNOWLEDGEMENT

The authors would like to thank Faculty of Pharmacy, Mahidol University for the financial support.

REFERENCES

1. WHO. Health situation in the South-East Asia Region 1994-1997. New Delhi: World Health Organization Regional Office for South-East Asia; 1999.
2. World Health Organization. World health statistics 2007. Geneva: WHO Press; 2007.
3. Siriraj Cancer Center, Faculty of Medicine, Siriraj Hospital. Tumor registry: Statistical report 2006. Bangkok: Mahidol University; 2006.
4. Glennie MJ, Johnson PWM. Clinical trials of antibody therapy. *Rev Immunol Today* 2000; 21: 403-10.
5. See HT, Kavanagh JJ, Hu W, *et al.* Targeted therapy for epithelial ovarian cancer: Current status and future prospects. *Inter J Gynecol Cancer* 2003; 13: 701-34.
6. Green MR. Targeting targeted therapy. *N Engl J Med* 2004; 350: 2191-3.
7. Bast Jr RC, Badgwell D, Lu Z, *et al.* New tumor markers: CA125 and beyond. *Inter J Gynecol Cancer* 2005; 15: 274-81.
8. Baselga J, Arteaga CL. Critical update and emerging trends in epidermal growth factor receptor targeting in cancer. *J Clin Oncol* 2005; 23: 2445-59.
9. Moongkardi P, Kaslungka S, Kosem N, *et al.* Cytotoxicity and apoptosis of ovarian and breast cancer cell lines induced by OVS1 monoclonal antibody and paclitexel. *Asian Pac J Allergy Immunol* 2003; 21: 31-41.
10. Neungton N, Moongkarndi P, Neungton S, *et al.* Establishment of OVS1 and OVS2 monoclonal antibodies recognizing human ovarian mucinous cystadenocarcinoma. *Asian Pac Allergy Immunol* 1995; 13: 47-53.
11. Mahabusarakam W, Wirayachitra P. Chemical constituents of *Garcinia mangostana*. *J Nat Prod* 1987; 50: 474-8.
12. Matsumoto K, Akao Y, Yi H, *et al.* Preferential target is mitochondria in α-mangostin-induced apoptosis in human leukemia HL60 cells. *Bioorg Med Chem* 2004; 12: 5799-806.
13. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; 65: 55-63.
14. Studzinski GP. Cell growth and apoptosis: a practical approach. New York: IRL Press; 1995.
15. Wolfe CAC, Hage DS. Studies on the rate and control of antibody oxidation by periodate. *Anal Biochem* 1995; 231: 123-30.

16. Tian WM, Zhang CL, Hou SP, *et al.* Hyaluronic acid hydrogel as Nogo-66 receptor antibody delivery system for the repairing of injured rat brain: *in vitro*. *J Control Release* 2005; 102: 13-22.
17. Springer CJ, Dowell R, Burke PJ, *et al.* Optimization of alkylating agents derived from phenol and aniline mustard: A new clinical candidate prodrug (ZD2767) for Antibody-Directed Enzyme Prodrug Therapy (ADEPT). *J Med Chem* 1995; 38: 5051-65.
18. Moongkarndi P, Kosem N, Kaslungka S, *et al.* Antiproliferation, antioxidation and induction of apoptosis by *Garcinia mangostana* (mangosteen) on SKBR3 human breast cancer cell line. *J Ethnopharmacol* 2004; 90: 161-6.
19. Moongkarndi P, Kosem N, Uawisetwathana U, *et al.* Antiproliferative activity, apoptotic induction and cell cycle arrest of α-mangostin on human ovarian cancer cells. The 7th JSPS/NRCT Joint Seminar "Recent Advances in Natural Product Research and Its Application". December 1-4, 2006, University of Toyama, Toyama, Japan.