Brief Report

DOI: 10.5582/bst.2011.v5.3.93

Affinity of anti-insulin-like growth factor I receptor antibody binding to the receptor altered by plant lectins

Yu Kusada[1,2], Yoko Fujita-Yamaguchi[1,*]

[1] *Department of Applied Biochemistry, Tokai University, Kanagawa, Japan;*
[2] *Present Address: Solution Development Department, PLM Division, FASOTEC Co., Ltd., Chiba, Japan.*

Summary **The binding ability of anti-insulin-like growth factor I receptor (IGF-IR) single-chain variable fragments (scFvs) to IGF-IR was measured in the presence of plant lectins. Combinations of concanavalin A (Con A), wheat germ agglutinin (WGA), or peanut agglutinin (PNA) and 1H7 or 3B7 anti-IGF-IR scFv/phage antibodies that were previously produced and characterized were used. WGA inhibited binding of both scFvs proteins to the receptor. PNA slightly enhanced the binding of 1H7 scFv and phage antibody to the receptor. Con A led to enhancement of 3B7 scFv-binding but had no effect in a test of phage antibodies and determination of kinetic parameters. The effect of lectins differed for scFvs and phage antibodies, implying that affinity altered by lectins is dependent upon the molecular structure of the antibodies. Results indicated that animal lectins may affect the affinity of therapeutic antibodies targeting cell membrane receptors *in vivo*.**

Keywords: Altered affinity, scFv, IGF-IR, lectin

1. Introduction

Insulin-like growth factor I receptor (IGF-IR) is a hetero-tetrameric glycoprotein that consists of two α subunits and two β subunits. The α subunit is completely extracellular whereas the β subunit is a transmembrane protein with a cytoplasmic tyrosine kinase domain. When ligands like IGF-I and IGF-II bind to the receptor, tyrosine kinase is autophosphorylated and activated, leading to activation of down-stream signaling molecules including mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3 kinase (PI3K). This leads to biological action like the induction of proliferation or inhibition of apoptosis (*1,2*).

IGF-IR is overexpressed in various types of tumors and is thus considered to be a target molecule for cancer therapy (*3*). Several strategies targeting IGF-IR have been developed, including IGF-IR specific antibodies (*4,5*), tyrosine kinase inhibitors (*6*), small interference RNA (*7*), and a dominant negative type of IGF-IR (*8*). Of these, anti-IGF-IR antibodies are probably the best strategy for cancer therapy. The current authors previously reported that a chimeric scFv-Fc consisting of mouse anti-IGF-IR single-chain variable fragments (scFv) and a human IgG_1 Fc domain inhibits tumor growth *in vivo*; this tumor inhibition was attributed to IGF-IR down-regulation (*9,10*). More recently, the current authors produced scFvs derived from original monoclonal antibodies (mAb) that either inhibits growth (1H7) or has a stimulatory effect (3B7) (*11,12*), and determined the affinity and epitope specificity of 1H7 and 3B7 scFvs (*13*). Several other researchers also reported on the action of anti-IGF-IR antibodies with an eye towards the development of anti-IGF-IR antibody therapeutics (*14-16*).

A variety of proteins that recognize carbohydrate moieties play important roles in animals. Mannan-binding lectin (MBL), L-ficolin, M-ficolin, and H-ficolin are all complement-activating soluble pattern recognition molecules that play critical roles in human innate immunity (*17*). MBL and ficolins are reported to play a role in the clearance of apoptotic cells (*18,19*). Galetins are a family of proteins that bind to

**Address correspondence to:*
Dr. Yoko Fujita-Yamaguchi, Department of Applied Biochemistry, Tokai University, 4-1-1 Kitakaname, Hiratsuka, Kanagawa 259-1292, Japan.
e-mail: yamaguch@keyaki.cc.u-tokai.ac.jp

the β-galactoside carbohydrate structure through their carbohydrate recognition domains (*20*). These proteins have been shown to be involved in multiple biological functions such as cell-matrix and cell-cell interactions, cell proliferation, cell differentiation, cellular transformation, or apoptosis mainly through the way in which they bind to specific ligands. Partridge *et al.* reported that galectin-3 (soluble protein) can bind to the carbohydrate structures of EGF and TGF-β receptors that are modified by a specific Golgi enzyme (*21*). The multivalence of galectin-3 can create a lattice formation of those receptors, resulting in delays of receptor removal by constitutive endocytosis. Therefore, the lattice ensures up-regulation of surface receptors and increased sensitivity to growth factors (*21*). Those reports strongly suggest that circulating carbohydrate binding molecules may modify functions of cell surface receptors by binding to carbohydrate moieties on the receptors.

Plant lectins are known to alter the relationship between hormones and their receptors on cell membranes. For example, Buxser *et al.* reported that the binding of nerve growth factor to human melanoma cell membranes significantly increased when binding was carried out in the presence of wheat germ agglutinin (WGA) (*22*) and Masnikosa *et al.* reported that binding of IGF-II to its receptor increased in the presence of WGA (*23*).

Based on these studies, a hypothesis, *i.e.*, that animal lectins affect the dynamics of therapeutic antibodies targeting cell membrane receptors *in vivo*, was put forward. Therefore, model experiments were performed to test whether anti-IGF-IR scFv affinity for IGF-IR changes in the presence of plant lectins. This study clearly demonstrated that plant lectins significantly affect scFvs affinity *in vitro*. The results of this study thus suggest that the affinity of therapeutic antibodies targeting receptors may be modified by circulating animal lectins. This important finding needs to be addressed when developing therapeutic antibodies.

2. Materials and Methods

2.1. *Materials*

Recombinant human extracellular domain of IGF-IR (rhIGF-IR) and insulin receptor (rhIR) were purchased from R&D Systems Inc. (MN, USA). Concanavalin A (Con A), WGA, and peanut agglutinin (PNA) labeled with biotin were obtained from Seikagaku Biobusiness (Japan). Horse radish peroxidase (HRP) conjugated with streptavidin, HRP-conjugated anti-E tag, and anti-M13 antibody were obtained from GE Healthcare (Piscataway, NJ, USA). 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS)/H_2O_2 was from Roche Diagnostics (Mannheim, Germany).

2.2. *Selection and preparation of lectins*

Three carbohydrate-binding plant lectins, Con A, WGA, and PNA, were used in this study. Con A is a homotetramer of 26.5 kDa-subunit with a binding site for α-D-mannosyl and α-D-glucosyl residues (*24*). WGA exists as a heterodimer with a molecular weight of approximately 38 kDa and selectively recognizes *N*-acetylglucosaminyl residues that are predominantly found on the plasma membrane (*25*). PNA is a homotetramer of approximately 27 kDa-subunit and is galactose-specific and had strong anti-T-antigen activity (*26*). Commercially available biotin-labeled lectins were dissolved in phosphate buffer saline pH 7.4 (PBS) according to the manufacturer's instructions to yield stock solutions with final concentrations of 1 mg/mL.

2.3. *Preparation of scFvs or phage antibodies*

Phage antibodies and scFvs derived were produced from mouse monoclonal antibodies as previously described (*13*). 1H7 and 3B7 were scFvs in a VL-VH orientation whereas 1H7R and 3B7R were scFvs in a VH-VL orientation. For preparation of phage antibodies, *E. coli* cells were cultured in 40 mL of SBSC at room temperature for 2 h, followed by infection with 8.8×10^{10} pfu M13KO7 helper phage for 1 h at 37ºC. Infected *E. coli* was selected by culturing in the presence of 50 μg/mL kanamycin at 25ºC for 2 d. The culture supernatants containing phages were precipitated in 4% polyethylene glycol/0.5 M NaCl (PEG precipitation) and resuspended in 20 mM Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl (TBS) with 1.5% BSA and 0.2% Tween-20 followed by treatment with benzonaze (Novagen) to digest any unnecessary DNA. The titer (cfu) of each phage was determined by means of infection activity against *E. coli* TG1 cells. Each resulting phage was subjected to ELISA.

For preparation of scFvs, *E. coli* cells were cultured overnight in 5 mL of 2 × YT medium containing 50 μg/mL carbenicillin, 50 μg/mL spectinomycin (2 × YTCS), and 1% glucose at 25ºC. The overnight culture was inoculated in 40 mL of freshly-prepared 2 × YTCS and incubated for 1 h at 30ºC, followed by induction with 1 mM IPTG at 30ºC for 5 h. Periplasmic fractions that contained soluble scFvs were collected by the osmotic shock method as described (*13*).

2.4. *Binding assay of lectins to rhIGF-IR or rhIR determined by ELISA*

Each well of a 96-well plate was coated with 250 ng/50 μL of rhIGF-IR or rhIR followed by incubation for 2 h at room temperature. Antigen-coated wells and control wells were blocked by incubation overnight

with 150 µL of 3% BSA/TBS at 4ºC. The wells were then incubated with 50 µL of various lectins labeled with biotin at room temperature for 2 h. The wells were washed 3 times with 150 µL of TBS containing 0.2% Tween-20 (TBST). For detection of lectins, HRP-conjugated streptavidin (1:1,000 dilution) was added to the wells and incubated at room temperature for 1 h. The wells were washed 7 times with TBST and then 3 times with TBS. Peroxidase activity was detected by reaction with 100 µL of ABTS/H_2O_2 for 30 min and termination with 1% oxalic acid. The absorbance at 415 nm was measured by a plate reader (Model 680; Bio-Rad Laboratories, Hercules, CA, USA).

2.5. *Affinity of scFvs or phage antibodies binding to rhIGF-IR altered by lectins*

Antigen-coated wells were prepared and blocked by BSA and incubated with Con A, WGA, or PNA as described above. ScFvs or phage antibodies were then added to the wells and incubated at room temperature for 2 h. The wells were washed 3 times with TBST. For detection of bound scFvs or phage antibodies, HRP-conjugated anti-E tag antibody or anti-M13 antibody, respectively, was added to the wells and incubated at room temperature for 1 h. Peroxidase activity was detected as described above.

2.6. *Determination of kinetic dissociation constants*

ELISA-based measurements of the dissociation constant (K_D) were performed as described previously (*26,27*). Briefly, scFvs were incubated with excess concentrations of antigens or antigens pre-incubated with Con A (1 µg/mL), WGA (100 µg/mL), or PNA (100 µg/mL) at 4ºC overnight. Equilibrated antibodies/antigen complexes were transferred to wells coated with antigens or antigens pre-incubated with WGA that were blocked with BSA. After incubation at room temperature for 1 h, the wells were washed and bound antibodies were detected by adding secondary antibodies as described above. The value of K_D was determined as previously described (*26,27*).

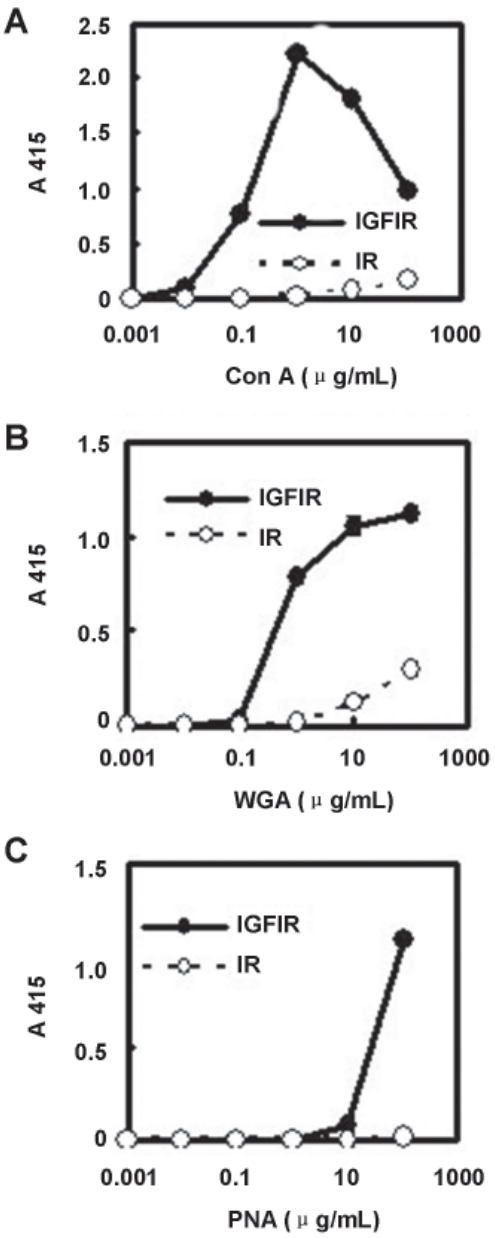

Figure 1. Binding assays of lectins to rhIGF-IR or rhIR as determined by ELISA. Binding of Con A **(A)**, WGA **(B)**, or PNA **(C)** to IGF-IR (●) or IR (○) was determined by ELISA as described in the "Materials and Methods". The error bars represent the standard deviation (n = 3).

3. Results and Discussion

First, the binding of three plant lectins to IGF-IR or IR was investigated. As shown in Figure 1, Con A (Figure 1A), WGA (Figure 1B), and PNA (Figure 1C) all bound to rhIGF-IR in a dose-dependent manner. Surprisingly, there was significantly little binding of three lectins to rhIR. This could be due to the recombinant IR used, which may differ from native IR in terms of glycosylation. Although the results of lectin binding to IGF-IR and IR were strikingly different, the positive results with IGF-IR were utilized to evaluate the effects of these lectins on scFv binding to rhIGF-IR.

Affinity of 1H7 and 3B7 scFvs in the presence of three concentrations of lectins was then examined. Con A did not significantly affect the affinity of scFvs except at 1 µg/mL (Figure 2A). WGA at 100 µg/mL significantly inhibited both 1H7 and 3B7R binding (Figure 2B). PNA at 10 and 100 µg/mL slightly enhanced 1H7 affinity but did not affect the binding of 3B7R scFv (Figure 2C).

To determine whether affinity altered by plant lectins depends on the molecular structure of antibodies, the same experiment as in Figure 2 was performed using phage antibodies expressing scFv proteins in two orientations – 1H7R or 3B7R in VH-VL

and 1H7 or 3B7 in VL-VH and lectin concentrations that affected the binding of 1H7 or 3B7R scFv protein to the receptor. With 1H7 and 1H7R phage antibodies, enhancement by PNA and inhibition by WGA were observed (Figures 3A and 3B) with 1H7 scFv (Figure 2). In contrast, slight enhancement by Con A and inhibition by WGA were observed with 3B7R phage antibody, but neither action was observed with 3B7 phage antibody (Figures 3C and 3D).

To further study the effects of lectins on the binding of scFv proteins to rhIGF-IR, affinity constants for binding of 1H7 and 3B7R scFv proteins to the receptor were determined in the presence or absence of the three lectins (Table 1). The control K_D values of 1H7 and 3B7R scFv in the absence of lectins were 16.4 ± 0.8 and 13.2 ± 2.0 nM, respectively. In the presence WGA, the K_D values of 1H7 and 3B7R scFv increased to 196 ± 40.0 and 41.0 ± 2.2 nM, respectively, indicating lower affinity. In the presence of PNA, the K_D value of 1H7 scFv slightly decreased to 9.31 ± 0.71 nM, indicating a slightly higher affinity. In the presence of Con A, however, the K_D value of 3B7R changed little from the control. These results coincide with those originally observed (Figure 2).

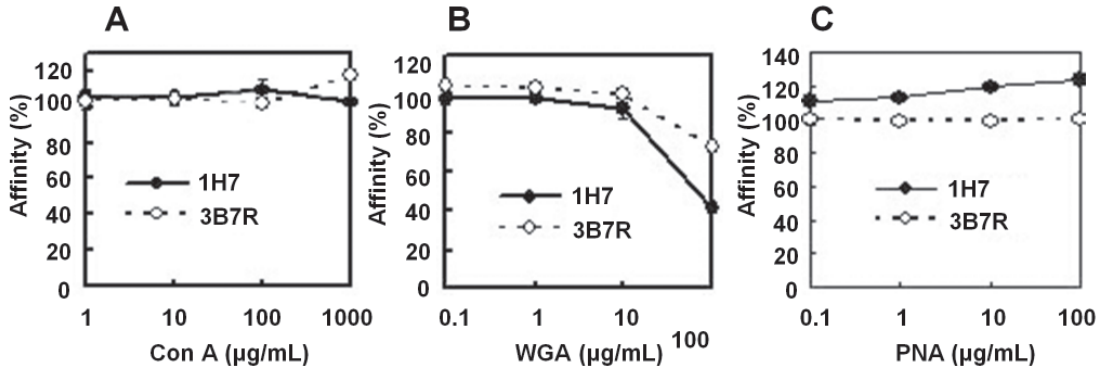

Figure 2. Alteration of anti-IGF-IR scFv protein binding to IGF-IR by lectins. Binding of 1H7 (●) or 3B7R (○) scFv proteins to rhIGF-IR in the presence of Con A **(A)**, WGA **(B)**, or PNA **(C)** at indicated concentrations was measured by ELISA as described in the "Materials and Methods". The error bars represent the standard deviation (n = 3).

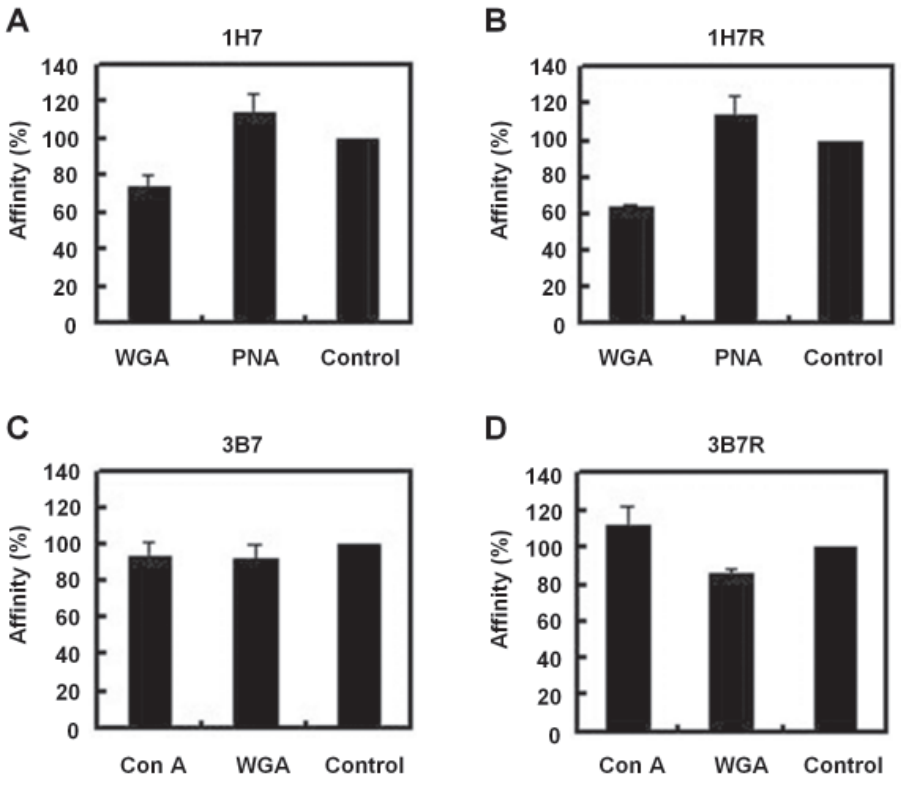

Figure 3. Alteration of anti-IGF-IR phage antibody binding to IGF-IR by lectins. Binding of 1H7 **(A)**, 1H7R **(B)**, 3B7 **(C)**, or 3B7R **(D)** phage antibodies to rhIGF-IR in the presence of Con A at 1 μg/mL, WGA at 100 μg/mL, or PNA at 100 μg/mL was measured by ELISA as described in the "Materials and Methods". The error bars represent the standard deviation (n = 3).

Table 1. Kinetic parameters of 1H7 and 3B7R scFvs in the presence or absence of lectins

Treatment	1H7 scFv	3B7R scFv
WGA (+)	196 ± 40.0	41.0 ± 2.2
PNA (+)	9.31 ± 0.71	ND
Con A (+)	ND	16.6 ± 1.7
Control	16.4 ± 0.8	13.2 ± 2.0

ND: Not determined. The dissociation constant (K_D, nM) was determined by ELISA as described in the "Materials and Methods".

This study demonstrated that plant lections that bind carbohydrate moieties on IGF-IR alter the affinity of anti-IGF-IR antibodies binding to the receptor. The most significant effect observed was that when the receptor was treated with WGA. WGA at 100 μg/mL significantly inhibited the binding of both 1H7 and 3B7 scFvs to the receptor. The inhibitory effect of WGA was also observed with 1H7, 1H7R, and 3B7R phage antibodies but not with 3B7 phage antibody to the receptor. A possible conformational difference due to the opposite orientation of VL and VH may have resulted in this difference. The presence of PNA slightly enhanced the binding of 1H7 scFv as well as 1H7 and 1H7R phage antibodies to the receptor. The changes in affinity observed in the presence of lectins could be due to changes in receptor conformation or steric hindrance. Con A slightly stimulated 3B7R scFv-binding to the receptor, which was confirmed by experiments with 3B7R phage antibodies and K_D measurements. Although this study used a model system, the results indicated that binding kinetics of therapeutic anti-IGF-IR antibodies to the receptor on cancer cells may be altered by animal lectins.

In conclusion, this *in vitro* study suggests that therapeutic antibodies targeting cell membrane receptors may be altered *in vivo* in terms of their affinity or action by circulating carbohydrate-binding proteins.

Acknowledgements

This work was supported by grants 1750120 and 22570125 from the Japan Society for the Promotion of Science and in part by a grant from JST CREST.

References

1. LeRoith D, Werner H, Beitner-Johnson D, Roberts CT Jr. Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocr Rev. 1995; 16:143-163.
2. LeRoith D, Roberts CT Jr. The insulin-like growth factor system and cancer. Cancer Lett. 2003; 195:127-137.
3. Haddad T, Yee D. Targeting the insulin-like growth factor axis as a cancer therapy. Future Oncol. 2006; 2:101-110.
4. Kusada Y, Fujita-Yamaguchi Y. An overview of currently available anti-insulin-like growth factor I receptor antibodies. Biosci Trends. 2007; 1:128-133.
5. Hofmann F, García-Echeverría C. Blocking the insulin-like growth factor-I receptor as a strategy for targeting cancer. Drug Discov Today. 2005; 10:1041-1047.
6. Hopfner M, Sutter AP, Huether A, Baradari V, Scherubl H. Tyrosine kinase of insulin-like growth factor receptor as target for novel treatment and prevention strategies of colorectal cancer. World J Gastroenterol. 2006; 12:5635-5643.
7. Neuenschwander S, Roberts CT Jr, LeRoith D. Growth inhibition of MCF7 breast cancer cells by stable expression of an insulin-like growth factor I receptor antisense ribonucleic acid. Endocrinology. 1995; 136:4298-4303.
8. Lee CT, Park KH, Adachi Y, Seol JY, Yoo CG, Kim YW, Han SK, Shim YS, Coffee K, Dikov MM, Carbone DP. Recombinant adenoviruses expressing dominant negative insulin-like growth factor-I receptor demonstrate antitumor effects on lung cancer. Cancer Gene Ther. 2003; 10:57-63.
9. Li SL, Liang SJ, Guo N, Wu AM, Fujita-Yamaguchi Y. Single-chain antibodies against human insulin-like growth factor I receptor: Expression, purification, and effect on tumor growth. Cancer Immunol Immunother. 2000; 49:243-252.
10. Sachdev D, Li SL, Hartell JS, Fujita-Yamaguchi Y, Miller JS, Yee D. A chimeric humanized single-chain antibody against the type I insulin-like growth factor (IGF) receptor renders breast cancer cells refractory to the mitogenic effects of IGF-I. Cancer Res. 2003; 63:627-635.
11. Li SL, Kato J, Paz IB, Kasuya J, Fujita-Yamaguchi Y. Two new monoclonal antibodies against the alpha subunit of the human insulin-like growth factor-I receptor. Biochem Biophys Res Commun. 1993; 196:92-98.
12. Xiong L, Kasuya J, Li SL, Kato J, Fujita-Yamaguchi Y. Growth-stimulatory monoclonal antibodies against human insulin-like growth factor I receptor. Proc Natl Acad Sci U S A. 1992; 89:5356-5360.
13. Kusada Y, Morizono T, Matsumoto-Takasaki A, Sakai K, Sato S, Asanuma H, Takayanagi A, Fujita-Yamaguchi Y. Construction and characterization of single-chain antibodies against human insulin-like growth factor-I receptor from hybridomas producing 1H7 or 3B7 monoclonal antibody. J Biochem. 2008; 143:9-19.
14. Burtrum D, Zhu Z, Lu D, *et al*. A fully human monoclonal antibody to the insulin-like growth factor I receptor blocks ligand-dependent signaling and inhibits human tumor growth *in vivo*. Cancer Res. 2003; 63:8912-8921.
15. Wang Y, Hailey J, Williams D, *et al*. Inhibition of insulin-like growth factor-I receptor (IGF-IR) signaling and tumor cell growth by a fully human neutralizing anti-IGF-IR antibody. Mol Cancer Ther. 2005; 4:1214-1221.
16. Goetsch L, Gonzalez A, Leger O, Beck A, Pauwels PJ, Haeuw JF, Corvaia N. A recombinant humanized anti-insulin-like growth factor receptor type I antibody (h7C10) enhances the antitumor activity of vinorelbine and anti-epidermal growth factor receptor therapy against human cancer xenografts. Int J Cancer. 2005; 113:316-328.
17. Thiel S. Complement activating soluble pattern recognition molecules with collagen-like regions, mannan-binding lectin, ficolins and associated proteins. Mol Immunol. 2007; 44:3875-3888.

18. Nauta AJ, Raaschou-Jensen N, Roos A, Daha MR, Madsen HO, Borrias-Essers MC, Ryder LP, Koch C, Garred P. Mannose-binding lectin engagement with late apoptotic and necrotic cells. Eur J Immunol. 2003; 33:2853-2863.
19. Matsushita M. Ficolins: Complement-activating lectins invovled in innate immunity. J Innate Immun. 2010; 2:24-32.
20. Nakahara S, Raz A. On the role of galectins in signal transduction. Methods Enzymol. 2006; 417:273-289.
21. Partridge EA, Roy CR, Guglielmo GMD, Pawling J, Cheung P, Granovsky M, Nabi IR, Wrana JL, Dennis JW. Regulation of cytokine receptors by golgi N-glycan processing and endocytosis. Science. 2004; 306:120-124.
22. Buxser SE, Kelleher DJ, Watson L, Puma P, Johnson GL. Change in state of nerve growth factor receptor. J Biol Chem. 1983; 258:3741-3749.
23. Masnikosa R, Baricević I, Jones DR, Nedić O. Characterisation of insulin-like growth factor receptors and insulin receptors in the human placenta using lectin affinity methods. Growth Horm IGF Res. 2006; 16:174-184.
24. Rüdiger H, Gabius H. Plant lectins: Occurrence, biochemistry, functions and applications. Glycoconj J. 2001; 18:589-613.
25. Nagata Y, Burger MM. Wheat germ agglutinin. Molecular characteristics and specificity for sugar binding. J Biol Chem. 1974; 249:3116-3122.
26. Ryder SD, Smith JA, Rhodes JM. Peanut lectin: A mitogen for normal human colonic epithelium and human HT29 colorectal cancer cells. J Natl Cancer Inst. 1992; 84:1410-1416.
27. Friguet B, Chaffotte AF, Djavadi-Ohaniance L, Goldberg ME. Measurements of the true affinity constant in solution of antigen-antibody complexes by enzyme-linked immunosorbent assay. J Immunol Methods. 1985; 77:305-319.
28. Stevens FJ. Modification of an ELISA-based procedure for affinity determination: Correction necessary for use with bivalent antibody. Mol Immunol. 1987; 24:1055-1060.

(Received March 30, 2011; Revised May 13, 2011; Accepted May 16, 2011)