DUAL ROLES OF CANCER CELL-EXPRESSED IMMUNOGLOBULINS IN CANCER IMMUNOLOGY

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ABSTRACT

While the expression of immunoglobulins and T cell receptors on cancer cells has been well-established for decades, the potential roles and mechanisms of action of these cancerous antigen receptors have not been fully elucidated. A monoclonal antibody designated as RP215, which reacts specifically with the carbohydrate-associated epitope located on the heavy chain region of cancerous immunoglobulins and T cell receptors, was used as a unique probe to study the roles of antigen receptors in the immunology of cancer cells. Through extensive cell-based biological and immunological studies, it was found that both anti-antigen receptors and RP215 demonstrated similar actions on the gene regulations involved in the growth/proliferation of cancer cells, as well as on toll-like receptors involved in innate immunity. In addition, RP215-specific cancerous immunoglobulins are believed to capture or neutralize circulating antigen/antibodies which may be harmful to cancer cells within the human body. In contrast to normal B and T cells and their respective receptors in the conventional immune system, cancer cells co-express both immunoglobulins and T cell receptors and immune protection is exercised by unique mechanisms. For example, these cancer cell-expressed antigen receptors display a lack of class switching, limited hyper-mutation, aberrant glycosylations and a strong influence on the toll-like receptors of cancer cells. Therefore, it is hypothesized that both normal and cancerous immune systems may co-exist and operate simultaneously within the human body. The balance of these two immune factors for respective surveillance and protection may be relevant to the outcome of cancer immunotherapy in humans. A potential therapeutic strategy is being developed by using RP215 as a drug candidate to target cancer cells based on these observations.

Keywords: Cancerous Immunoglobulins, CA215, RP215 Monoclonal Antibody, Two Immune Systems, Cancer Immunology

1. INTRODUCTION

The expressions of antigen receptors, such as immunoglobulins and T cell receptors, in cancer cells were not discovered until the 1990s (Babbage *et al*., 2006; Chen and Gu, 2007; Hu *et al*., 2011; 2012; Huang *et al*., 2008; 2009; Kimoto, 1998; Lee and Ge, 2009; Lee *et al*., 2008; Li *et al*., 2001; 2004; Qiu *et al*., 2003; Zhang *et al*., 2012; Zheng *et al*., 2007a; 2007b; 2009; Zhu *et al*., 2008). In the traditional immune system, it has been well-established that both B and T lymphocytes play differential and interacting roles in our common immune responses against foreign pathogens, such as bacteria and viruses, in the normal human body

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(Murphy *et al*., 2007). In comparison, the mechanisms of action of antigen receptors widely expressed on the cancer cell surface are not fully understood and require further investigations (Kimoto, 1998).

Based on previous cell-based bioassays with target-specific small interfering Ribonucleic Acid (siRNA) experiments, it was generally concluded that the expressions of cancerous immunoglobulins are essential for the growth/proliferation of cancer cells *in vitro* and *in vivo* (Li *et al*., 2004; 2012; Qiu *et al*., 2003). However, it remains to be established if the expressions of antigen receptors on the cancer cell surface play any other functional roles in the immunology of cancer cells, including immune protection or evasion (Vince *et al*., 2007).

A major breakthrough came through in 1987, when a monoclonal antibody designated as RP215 was generated against the cell extract of OC-3-VGH ovarian cancer cell line (Lee *et al*., 1992). It was later established that RP215 reacts specifically with the carbohydrate-associated epitope located mainly on the heavy chains of cancerous immunoglobulins and cancerous T cell receptors and not with antigen receptors of the normal immune system (Lee *et al*., 2006). The group of RP215-specific glycoproteins was termed in general as CA215 and consists mainly of immunoglobulin super family proteins (Lee *et al*., 2012b). Therefore, RP215 was used as the unique probe for cancerous antigen receptors to study their specific/distinct functional roles in cancer cells. One can then ask if such a cancer immune system has evolved to exist and is being operated independently of our known normal immune system in the human body (Liu *et al*., 2010).

Since the unexpected discovery of RP215, many biological and immunological studies have been performed during the last decade to investigate the roles of cancerous antigen receptors and elucidate their mechanisms of action in cancer cells (Lee *et al*., 1992). We believe that the results of these studies may lead to a better strategy in the immunotherapy of cancer cells in humans.

2. STRUCTURAL COMPARISONS BETWEEN NORMAL AND CANCEROUS IMMUNOGLOBULINS

By the use of Reverse Transcription Polymerase Chain Reaction (RT-PCR), it was confirmed that the primary amino acid sequences of normal human IgG and cancer cell-expressed IgG (C-IgG(are virtually identical (Lee *et al*., 2006). This would indicate that the genes involved in cancerous immunoglobulins and those of normal immunoglobulins are of the same origin in humans (Zheng *et al*., 2009).

By Western blot assay with OC-3-VGH cancer cell extract and with affinity-purified CA215, RP215 was shown to react with broad protein bands with a molecular size ranging from 50 to 70 KDa (Lee *et al*., 2008; 2006). Through Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) analysis, the identity of these affinity-purified CA215 was determined to be mainly the heavy chains of immunoglobulins (Lee *et al*., 2008; 2012b).

A sandwich immunoassay using RP215 as the capturing antibody and horseradish peroxidase (HRP)-conjugated RP215 as the detecting antibody was employed to generate dose-dependent signals of CA215 (**Fig. 1A**). In this assay, it was also demonstrated that goat anti-human IgG-Fc antibodies (1 µg mL^{-1}) showed little inhibitions on CA215 signals. This is in contrast to goat anti-human IgG-Fab, where the binding between RP215 and CA215 was greater than 95% inhibited. Results of such comparative binding assays suggested that the RP215-specific "sugar" epitope is localized preferentially in the Fab region of the heavy chain of C-IgG and not in the Fc region (Lee and Azadi, 2012; Lee *et al*., 2008).

Tunicamycin is known to be a potent inhibitor of N-glycosylation in human cells (Gonzales *et al*., 2006). No inhibition of RP215 binding to cancer cells was observed in the presence of high doses of tunicamycin within the cancer cell culture (**Fig. 1B**). This observation would indicate that the carbohydrate-associated epitope recognized by RP215 is not N-glycan-linked, but O-linked. Extensive glycoanalysis was performed with affinity-purified CA215, including CA215-D, which was obtained through a further purification with goat anti-human IgG affinity column. The preliminary results of O-linked glycans associated with CA215 are presented in **Table 1** (Lee and Azadi, 2012). Similar O-linked glycan structures were detected when those of CA215 obtained from OC-3-VGH ovarian and C33A cervical cancer cell lines was compared, respectively. From this glycoanalysis, several O-linked oligoglycans were consistently detected. The details of the elucidated structural linkage relationships are still being worked out (Lee and Azadi, 2012).

3. CLASS SWITCHING AND SOMATIC HYPERMUTATION OF NORMAL AND CANCEROUS IMMUNOGLOBULINS

Normal immunoglobulins are expressed through specific class switching processes and serial somatic hypermutations (Honjo *et al*., 2002; Muramatsu *et al*., 2000; Papavasiliou and Schatz, 2002). In contrast, cancerous immunoglobulins can be produced from a single clone with many classes and subclasses expressed simultaneously (Early *et al*., 1980; Honjo *et al*., 2002). Based on the molecular cloning analysis, a limited diversity of immunoglobulins with restricted somatic mutations ($\leq 20\%$) was found to be expressed in cancer cells (Early *et al*., 1980; Honjo *et al*., 2002). The octamer element-2 (5'-ATGCAAT-3') (Oct-2) located in the immunoglobulin promoter region was determined to be crucial to cancerous IgG expression (Zhu *et al*., 2010).
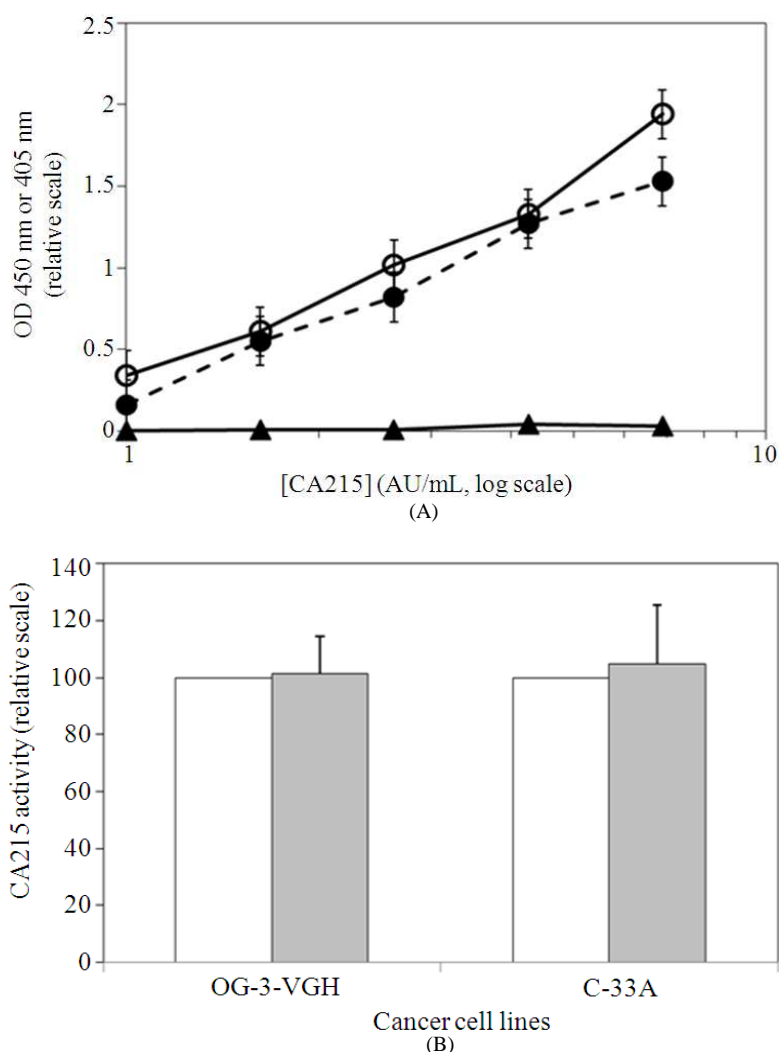

Fig. 1. Activity of CA215. (A): RP215-based enzyme immunoassay to reveal dose-dependent signal of CA215 (○) (expressed in AU/ml) and ef-fects of goat anti-human IgG-Fc (●) (1 µg mL^{-1}) and anti-human IgG-Fab (▲) (1 µg mL^{-1}) on CA215 signal intensity. Details of a typical RP215 enzyme immunoassay are described previously (Lee and Ge, 2009; Lee *et al*., 2008). (B): Effects of 48hr tunicamycin treatments (1 µg mL^{-1}) on CA215 activity in the supernatant of two cultured cancer cells for OC-3-VGH and C-33A. To each cell line the left column is the negative control (assuming 100% activity) without drug-treatment and the right column is the relative CA215 activity following the drug treatment (expressed in triplicate)

Table 1. Comparative profiles of permethylated O-linked glycans of five different CA215 samples which consist mainly of cancerous IgG

Sample ID	Observed Mass m/z [M+Na]+	Proposed structure	Structure
CA215 (lots: A, B and C)[a]	534	GalNAc1Gal1	
CA215 (lots: A and B)	708	GalNAc1Gal1Fuc1	
CA215 (lots: A, B, D, C, E and F)[b]	896	GalNAc1Gal1NeuAc1	
CA215 (lots: C, E and F)	926	GalNAc1Gal1NeuGc1	
CA215C	940[c]	GalNAc1GlcNAc1NeuAc1	
CA215 (lots: A, B and C)	1140	GalNAc1GlcNAc1Gal1NeuAc1	
CA215 (lots: C, D, E and F)[b]	1257	GalNAc1Gal1NeuAc2	
CA215 (lots: C, E and F)	1317	GalNAc1Gal1NeuGc2	
CA215 (lots: A, B, C, E and F)	1345	GalNAc1GlcNAc1Gal2NeuAc1	
CA215 (lots: C, E and F)	1375	GalNAc1GlcNAc1Gal2NeuGc1	

[a]CA215 lots A, B and C were from OC-3-VGH ovarian cancer cells lots A and B were obtained through acid elution, whereas lots C, D, E and F were obtained through elution with 3M urea. [b]Lot CA215D was obtained by an additional purification of urea-eluted CA215 with goat anti-human IgG affinity column followed by the same analysis (CA215D is designated as affinity-purified cancerous IgG). CA215 lots E and F were from C-33A cervical cancer cells. [c]Detected by MALDI-TOF MS method but not found by NSI-MS method. [d]N-acetylgalactosamine (□), N-acetylglucosamine (■), Fucose (▲), Galactose (●), N-acetylneuraminic acid (♦) and N-glycolylneuraminic acid (♦). Obtained from (Lee and Azadi, 2012) with permission

In contrast, octamer element-1 (Oct-1) is essential in normal B cells and is used as transcriptional factor (Zheng *et al*., 2009). These observations show that the class switching phenomenon, which occurs in normal B cells, does not take place in immunoglobulins expressed by cancer cells. There is also little diversity of immunoglobulins expressed by cancer cells due to limited somatic hypermutations in the cancerous immune system (Early *et al*., 1980; Honjo *et al*., 2002; Zheng *et al*., 2009).

The molecular nature of cancerous immunoglobulin genes has been previously explored (Zheng *et al*., 2009). For example, seven dominant specific restricted patterns of $V_{H\gamma}DJ_{H\gamma}$ recombination have been found in cancerous immunoglobulins. However, the mechanisms of hypermutation were different from antigen selection in the B-cell-derived $V_{H\gamma}DJ_{H\gamma}$ sequence. Therefore, a distinct repertoire of $V_{H\gamma}DJ_{H\gamma}$ must exist for cancerous immunoglobulins.

4. GENE REGULATION STUDIES OF CANCEROUS IMMUNOGLOBULINS

Attempts were made to study the changes in gene expression of cultured cancer cells in response to challenge by RP215, anti-human IgG, or anti-T-cell receptors (Zhou *et al*., 2006). Among these were genes involved in the regulation of the cell cycle (cyclin D1, P21, c-fos), protein synthesis (ribosomal proteins P_0, P_1), regulation of growth (Epidermal Growth Factor Receptor (EGFR), IgG, T Cell Receptor (TCR), Nuclear Factor kappa-B p105 subunit 1 (NFκB-1)) and Toll-Like Receptors (TLR-2, 3, 4, 9), which are essential for the innate immunity of cancer cells (Tang *et al*., 2013). Based on the results of these extensive studies with semi-quantitative RT-PCR, efforts were made to analyze if the expressions or regulations of these genes are correlated by the presence of any of these three anti-antigen receptor ligands (Zhou *et al*., 2006).

The results of such comparative gene expression analysis are presented in **Table 2** (Vince *et al*., 2007; Zhou *et al*., 2006). A high degree of consistency in the changes on gene regulation by these three anti-antigen receptors was observed. The correlation coefficients range from 0.90-0.95 when the pair-wise statistical analyses are performed with either OC-3-VGH ovarian or C-33A cervical cancer cell line as the testing model. Among these genes, those of toll-like receptors were strongly affected by RP215 or any other of the two anti-antigen receptors (Zhou *et al*., 2006). The results of these gene regulation studies seem to suggest that NFκB-1 gene is highly involved in the regulation of these genes involved in the growth/proliferation of cancer cells and

therefore is crucial to the immunology of cancer cells (Ditsworth and Zong, 2004; Gellman, 1997; Rokach *et al*., 1980; Xie *et al*., 2009; Young, 2012).

5. DETECTION OF BINDING SUBSTANCES OF CA215 OR CANCEROUS IMMUNOGLOBULINS IN POOLED HUMAN SERUM SPECIMENS

Attempts were made to identify any binding proteins/components exhibiting affinity to CA215 or C-IgG. Purified CA215 and/or cancerous immunoglobulins were used as the ligand to capture binding substances or proteins in human circulation (Lee *et al*., 2014a). The affinity-purified proteins isolated from pooled human serum (CA215 S+) were coated on microwells (**Fig. 2**). Biotinylated CA215 or cancerous IgG were shown to exhibit dose-dependent binding relationship to CA215 and C-IgG by Enzyme Immunoassay (EIA). The presence of CA215 or C-IgG-associated binding proteins or substances in pooled human serum specimens was clearly confirmed by binding ELISA in this study.
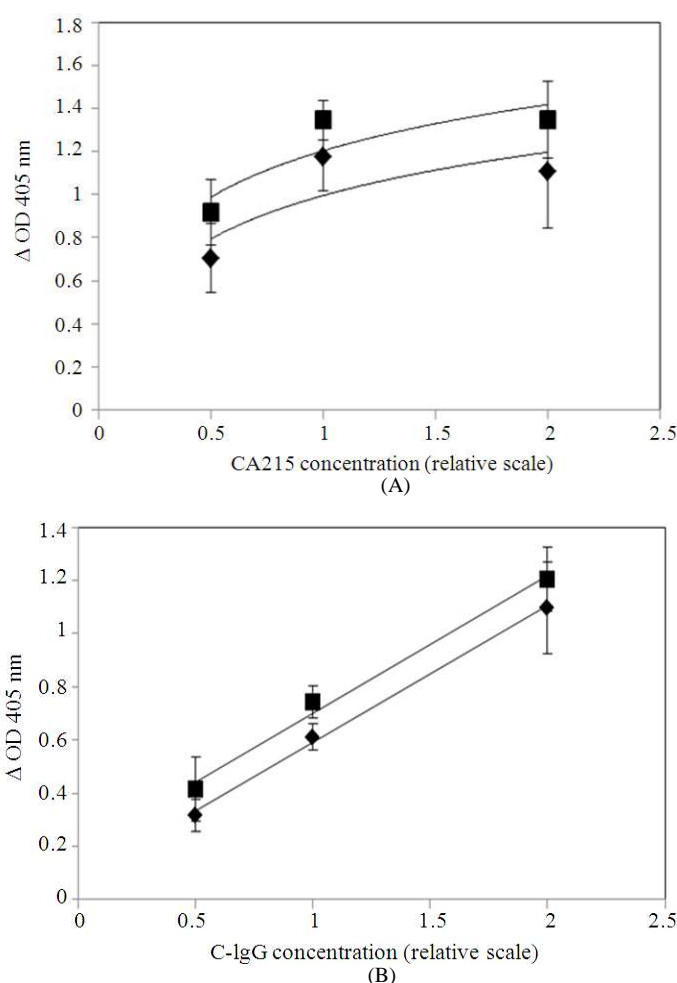

Fig. 2. Dose-dependent EIA binding activity between CA215 or C-IgG and microwells coated with affinity-purified binding proteins isolated from pooled human serum specimens. Affinity purified CA215 or C-IgG were first biotinylated prior to EIA binding studies. (A): Biotinylated CA215 versus C-IgG S+ coated wells (■), or CA215 S+ coated wells (♦). The signal intensity was determined with 3hr binding incubation at 37°C, then for signal detection, alkaline-phosphatase-labeled avidin was used for additional one hour incubation at 37 °C, followed by adding substrate for signal detection. Details were described previously (Lee *et al*., 2014a). (B): Biotinylated C-IgG versus C-IgG S+ coated wells (■), or CA215 S+ coated wells (♦). Experimental conditions are the same as those in (A)

Table 2. Effects of treatments of cultured OC-3-VGH ovarian or C-33A cervical cancer cells with Goat anti-human IgG (GαhIgG), rabbit anti-T Cell Receptors β (RαTCRβ) and murine RP215 (mRP215) on the expressions of genes involved in cell prolife-ration, protein synthesis, cell cycle regulations and the innate immunitya

Gene	GαhIgG		RαTCRβ		mRP215	
	OC-3-VGH	C-33A	OC-3-VGH	C-33A	OC-3-VGH	C-33A
IgG	0	▲▲▲	0	▲▲▲	0	▲▲▲
T cell receptor	△	0	△△△	▲	△△△	0
NFκB-1	△△△	▲▲▲	△△△	▲▲▲	△△△	▲▲▲
Cyclin D1	▽	▼▼	▽▽	▼	▽▽▽	▲▲▲
P21	△△△	▲	△△△	0	△△△	▲
c-fos	▽	▲▲▲	0	0	▽	▼▼
P_o	△△△	NA	▽▽▽	NA	0	NA
P_1	△△	▲	△	▲▲	△	▲▲▲
EGFR	△△△	NA	▽▽▽	NA	▽▽▽	NA
TLR-2	NA	▲▲	NA	▲	NA	▲▲▲
TLR-3	△△△	▲▲▲	△△△	▲▲▲	△△△	▲▲▲
TLR-4	▽▽▽	▼▼▼	▽▽▽	▼	▽▽▽	▼▼▼
TLR-9	▽▽▽	▼▼▼	▽▽▽	▼▼	▽▽▽	▼▼▼

[a](0) less than 10% gene expression increase or decrease, (△ or ▲) 10-20% gene expression increase, (△△ or ▲▲) 20-30% gene expression increase, (△△△ or ▲▲▲) above 30% gene expression increase, (▽ or ▼) 10-20% gene expression decrease, (▽▽ or ▼▼) 20-30% gene expression decrease, (▽▽▽ or ▼▼▼) above 30% gene expression decrease, (NA) not available. Modified from (Tang *et al*., 2013) with permission

These binding proteins could be of antibody or antigen origins or of other origins (Lee *et al*., 2014a). Based on these observations, it seems reasonable to assume that cancerous immunoglobulins or CA215 displayed on the cancer cell surface may have functional activities to capture or neutralize unidentified substances in pooled human serum specimens as a means of immune protection for the cancer cells (Lee *et al*., 2014a). Conversely, these CA215 or C-IgG-specific substances might be present in normal human circulation to neutralize or inactivate cancer cells. The reasons for the presence of these binding substances remain to be investigated. It is currently unknown whether these substances are naturally occurring or induced through appropriate immune responses to circulating cancer cells in the human immune system.

6. BIOLOGICAL AND IMMUNOLOGICAL ACTIVITITES OF RP215 OR OTHER ANTI-ANTIGEN RECEPTORS ON CANCER CELLS

Since C-IgGs are absolutely required for the growth/proliferation or survival of cancer cells *in vitro* or *in vivo*, RP215 or anti-antigen receptors were used as a probe in induced apoptosis studies in cultured cancer cells to demonstrate RP215's anti-proliferative actions (Chen and Gu, 2007; Lee *et al*., 2012a; Li *et al*., 2012;

Qiu *et al*., 2003; Zheng *et al*., 2007a). Results of such studies are presented in **Fig. 3A**. Through these studies, it was demonstrated that apoptosis can be induced in cultured cancer cells by a number of anti-antigen receptors, including RP215, anti-IgG, anti-IgM, anti-IgA, anti-Igλ and anti-TCR-β. Interestingly, anti-TLR-4 was also found to induce apoptosis in cultured cancer cells following the same incubation conditions. Similarly, RP215 was also shown to induce Complement-Dependent Cytotoxicity (CDC) reactions (Lee *et al*., 2012a). CDC reactions can also be induced in cancer cells upon incubation with many anti-antigen receptors. The results are shown in **Fig. 3B** for comparative purposes.

The results of these two cell-based biological assays clearly demonstrate that RP215 has anti-cancer properties and its humanized forms demonstrate the potential to be developed as anti-cancer drugs in humans for future therapeutic applications.

7. HYPOTHESIS OF TWO INDEPENDENT IMMUNE SYSTEMS AND THE DUAL ROLES OF CANCEROUS IMMUNOGLOBULINS

In this review, experimental observations have been summarized and presented to indicate that two independent immune systems may exist in the human body (Lee *et al*., 2014a).
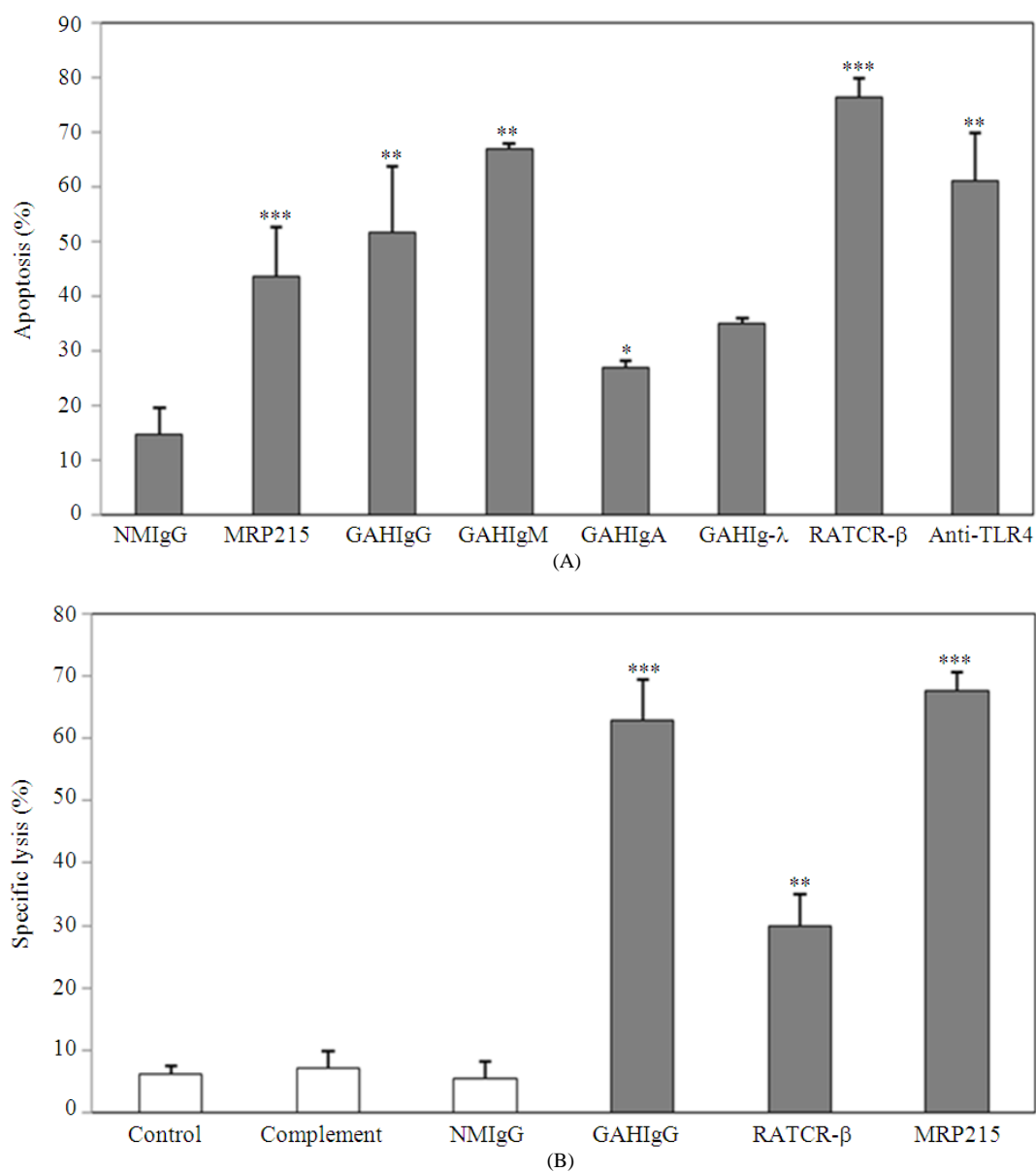
Fig. 3. Induced apoptosis of OC-3-VGH cancer cells. (A): Induced apoptosis on treated cultured OC-3-VGH cancer cells by Normal Mouse IgG (NMIgG) (Lane 1), Murine RP215 (MRP215) (Lane 2), (Lane 3), Goat Anti-Human IgM (GAHIgM) (Lane 4), Goat Anti-Human IgA (GAHIgA) (Lane 5), Anti-Human λ light Goat Anti-Human IgG (GAHIgG) chain (GAHIgλ) (Lane 6), Rabbit anti-T cell receptor β (RATCR-β) (Lane 7) and anti-TLR4 (Lane 8) for 24 h incubation. 10 µg mL^{-1} of the ligands were added to cancer cell culture medium for the apoptosis assay, by using terminal deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) assay kit from Roche Diagnostics (Lee *et al*., 2012a). (B): The Complement Dependent Cytotoxicity (CDC) assay to demonstrate the effect of goat anti-human IgG, anti-T cell receptor β and RP215 on complement-dependent cytotoxicty of OC-3-VGH ovarian cancer cell line; Lane 1: no treatment (control), Lane 2: complement only, Lane 3: Normal Mouse IgG and complement (NMIgG), Lane 4: Goat Anti-Human IgG and complement (GAHIgG), Lane 5: Rabbit Anti-T Cell Receptor β and complement (RATCR-β), Lane 6: Murine RP215 and complement (MRP215). *, **, *** indicate statistical significance of p<0.05, p<0.01 and p<0.001, respectively. Both (A) and (B) were modified from (Lee *et al*., 2012a) with permission

One is the common normal immune system, in which B and T lymphocytes play major roles in generating immunoglobulins and T cell receptors of high diversity through a series of interactions, differentiations and productions of effector molecules, including antibodies. An unlimited number of antibodies can be produced and released into circulation to fight off foreign pathogens such as viruses, fungi and bacteria (Lee *et al.*, 2014b, Murphy *et al.*, 2007; Lee *et al.*, 2014a). Our recent studies show that CA215 or CIgG-specific binding proteins can be detected in pooled human serum specimens and react with cancerous immunoglobulins (Lee *et al.*, 2014a). It remains to be demonstrated if these binding proteins or substances are antigens, antibodies, or other molecules which may be induced or occurring naturally (Lee *et al.*, 2014a).

Cancer cells might have their own immune system which can be distinguished from the conventional immune system within the human body. As mentioned previously, normal immunoglobulins and their cancerous counterparts can be differentiated by comparison of their mutual glycosylation patterns, class switching mechanisms, hypermutation, influences on innate immunity and apparent immunoactivity. Therefore, it is reasonable to assume that a separate independent immune system should be available for the growth/proliferation and immune protections of cancer cells under our normal human body environment. In fact, the existence of two separate immune systems is quite efficient and logical from an evolutionary point of view. Since cancer cells are recognized as "foreign" in our human body, the cancer immune system is one of the most effective means for immune protection and growth/proliferation of cancer cells. Any strategy resulting in the deactivation of this cancer immune system should lead to effective immunotherapy of relevant cancer cells in humans. Therefore, the widespread expressions of these cancerous immunoglobulins can be a potential target for cancer immunotherapy.

8. ACKNOLWEDGMENTS

9. REFERENCES

Babbage, G., C. Ottensmeier, J. Blaydes, F. Stevenson and S. Sahota, 2006. Immunoglobulin heavy chain locus events and expression of activation-induced cytidine deaminase in epithelial breast cancer cell lines. Cancer Res., 66: 3996-4000. DOI: 10.1158/0008-5472.CAN-05-3704

Chen, Z. and J. Gu, 2007. Immunoglobulin g expression in carcinomas and cancer cell lines. FASEB J., 21: 2931-2938. DOI: 10.1096/fj.07-8073

Ditsworth, D. and W.X. Zong, 2004. Nf-kappab: Key mediator of inflammation-associated cancer. Cancer Biol. Therapy, 3: 1214-1216. PMID: 15611628.

Early, P., H. Huang, M. Davis, K. Calame and L. Hood, 1980. An immunoglobulin heavy chain variable region gene is generated from three segments of DNA: Vh, d and jh. Cell Divis. Biol. California Institute Technol. Pasadena, 19: 981-992. DOI: 10.1016/0092-8674(80)90089-6

Gellman, S.H., 1997. Introduction: Molecular recognition. Chem. Rev., 97: 1231-1232. DOI: 10.1021/cr970328j

Gonzales, D., S.I. Rennard, M. Nides, C. Oncken and K.R. Reeves *et al.*, 2006. Varenicline, an α4β2 nicotinic acetylcholine receptor partial agonist, vs sustained-release bupropion and placebo for smoking cessation: A randomized controlled trial. J. Am. Med. Associat., 296: 47-55. DOI: 10.1001/jama.296.1.47

Honjo, T., K. Kinoshita and M. Muramatsu, 2002. Molecular mechanism of class switch recombination: Linkage with somatic hypermutation. Ann. Rev. Immunol., 20: 165-196. DOI: 0.1146/annurev.immunol.20.090501.112049

Hu, D., Z. Duan, M. Li, Y. Jiang and H. Liu *et al.*, 2011. Heterogeneity of aberrant immunoglobulin expression in cancer cells. Cellular Molecular Immunol., 8: 479-485. DOI: 10.1038/cmi.2011.25

Hu, F., L. Zhang, J. Zheng, L. Zhao and J. Huang *et al.*, 2012. Spontaneous production of immunoglobulin m in human epithelial cancer cells. PLoS One, 7: e51423-e51423. DOI: 10.1371/journal.pone.0051423

Huang, J., X. Sun, Y. Mao, X. Zhu and P. Zhang *et al.*, 2008. Expression of immunoglobulin gene with classical v-(d)-j rearrangement in mouse brain neurons. Int. J. Biochem. Cell Biol., 40: 1604-1615. DOI: 10.1016/j.biocel.2007.12.004

Huang, J., L. Zhang, T. Ma, P. Zhang and X. Qiu, 2009. Expression of immunoglobulin gene with classical v-(d)-j rearrangement in mouse testis and epididymis. J. Histochem. Cytochem., 57: 339-349. DOI: 10.1369/jhc.2008.951434

Kimoto, Y., 1998. Expression of heavy-chain constant region of immunoglobulin and t-cell receptor gene transcripts in human non-hematopoietic tumor cell lines. Genes Chromosomes Cancer, 22: 83-86. DOI: 10.1002/(sici)1098-2264(1998)22:1<83::aid-gcc12>3.0.co;2-o

Lee, C.Y., K.W. Chen, F.S. Sheu, A. Tsang and K.C. Chao *et al*., 1992. Studies of a tumor-associated antigen, cox-1, recognized by a monoclonal antibody. Cancer Immunol. Immunotherapy, 35: 19-26. DOI: 10.1007/BF01741050

Lee, G. and B. Ge, 2009. Cancer cell expressions of immunoglobulin heavy chains with unique carbohydrate-associated biomarker. Cancer Biomarkers, 5: 177-188. DOI: 10.3233/cbm-2009-0102

Lee, G. and P. Azadi, 2012. Peptide mapping and glycoanalysis of cancer cell-expressed glycoproteins ca215 recognized by rp215 monoclonal antibody. J. Carbohydrate Chem., 31: 10-30. DOI: 10.1080/07328303.2011.626544

Lee, G., A. Cheung, B. Ge, M. Zhu and B. Giolma *et al*., 2012a. Ca215 and gnrh receptor as targets for cancer therapy. Cancer Immunol. Immunotherapy, 61: 1805-1817. DOI: 10.1007/s00262-012-1230-8

Lee, G., M. Zhu, B. Ge and S. Potzold, 2012b. Widespread expressions of immunoglobulin superfamily proteins in cancer cells. Cancer Immunol., Immunotherapy, 61: 89-99. DOI: 10.1007/s00262-011-1088-1

Lee, G., C.Y. Huang, Y. Tang and H. Zhang, 2014a. Potential roles of cancerous immunoglobulins in the immunology of cancer cells. J. Clin. Cellular Immunol., 5: 1-7. DOI: 10.4172/2155-9899.1000200

Lee, G., C.Y. Huang, H. Zhang and Y. Tang, 2014b. The relationships between toll-like receptors and rp215-associated immunoglobulins expressed by cancer cells. J. Cancer Sci. Therapy, 6: 77-80. DOI: 10.4172/1948-5956.1000252

Lee, G., E. Laflamme, C.H. Chien and H.H. Ting, 2008. Molecular identity of a pan cancer marker, ca215. Cancer Biol. Therapy, 7: 2007-2014. DOI: 10.4161/cbt.7.12.6984

Lee, G., Q. Wu, C.H. Li, H.H. Ting and C.H. Chien, 2006. Recent studies of a new carbohydrate-associated pan cancer marker, ca215. J. Clin. Ligand Assay, 29: 47-51.

Li, J., C. Tan, Q. Xiang, X. Zhang and J. Ma *et al*., 2001. Proteomic detection of changes in protein synthesis induced by ngx6 transfected in human nasopharyngeal carcinoma cells. J. Prot. Chem., 20: 265-271. DOI: 10.1023/a:1010912311564

Li, M., D.Y. Feng, W. Ren, L. Zheng and H. Zheng *et al*., 2004. Expression of immunoglobulin kappa light chain constant region in abnormal human cervical epithelial cells. Int. J. Biochem. Cell Biol., 36: 2250-2257. DOI: 10.1016/j.biocel.2004.03.017

Li, M., H. Zheng, Z. Duan, H. Liu and D. Hu *et al*., 2012. Promotion of cell proliferation and inhibition of adcc by cancerous immunoglobulin expressed in cancer cell lines. Cellular Molecular Immunol., 9: 54-61. DOI: 10.1038/cmi.2011.40

Liu, J.Z., F. Tozzi, D.M. Waterworth, S.G. Pillai and P. Muglia *et al*., 2010. Meta-analysis and imputation refines the association of 15q25 with smoking quantity. Nature Genet., 42: 436-440. DOI: 10.1038/ng.572

Muramatsu, M., K. Kinoshita, S. Fagarasan, S. Yamada and Y. Shinkai *et al*., 2000. Class switch recombination and hypermutation require activation-induced cytidine deaminase (aid), a potential rna editing enzyme. Cell, 102: 553-563. DOI: 10.1016/s0092-8674(00)00078-7

Murphy, K., P. Travers and M. Walport, 2007. Janeway's immunobiology. 1st Edn., Garland Science, New York, ISBN-10: 9780815341239, pp: 323-496.

Papavasiliou, F.N. and D.G. Schatz, 2002. Somatic hypermutation of immunoglobulin genes: Merging mechanisms for genetic diversity. Cell, 109: 35-44.

Qiu, X., X. Zhu, L. Zhang, Y. Mao and J. Zhang *et al*., 2003. Human epithelial cancers secrete immunoglobulin g with unidentified specificity to promote growth and survival of tumor cells. Cancer Res., 63: 6488-6495.

Rokach, J., Y. Girard, Y. Guindon, J.G. Atkinson and M. Larue *et al*., 1980. The synthesis of a leukotriene with srs-like activity. Tetrahedron Lett., 21: 1485-1488. DOI: 10.1016/S0040-4039(00)92753-9

Tang, Y., H. Zhang and G. Lee, 2013. Similar gene regulation patterns for growth inhibition of cancer cells by rp215 or anti-antigen receptors. J. Cancer Sci. Therapy, 5: 200-208. DOI: 10.4172/1948-5956.1000207

Vince, J.E., W.W.L. Wong, N. Khan, R. Feltham and D. Chau *et al*., 2007. Iap antagonists target ciap1 to induce tnfα-dependent apoptosis. Cell, 131: 682-693. DOI: 10.1016/j.cell.2007.10.037

Xie, W., Y. Wang, Y. Huang, H. Yang and J. Wang *et al*., 2009. Toll-like receptor 2 mediates invasion via activating nf-kappab in mda-mb-231 breast cancer cells. Biochem. Biophysical Res. Commun., 379: 1027-1032. DOI: 10.1016/j.bbrc.2009.01.009

Young, R.N., 2012. Discovery and Development of Montelukast (singulair®). In: Case Studies in Modern Drug Discovery and Development, Huang, X. and R.G. Aslanian (Eds.)., John Wiley and Sons, Hoboken, ISBN-10: 1118219708, pp: 528.

Zhang, L., S. Hu, C. Korteweg, Z. Chen and Y. Qiu *et al*., 2012. Expression of immunoglobulin g in esophageal squamous cell carcinomas and its association with tumor grade and ki67. Human Pathol., 43: 423-434. DOI: 10.1016/j.humpath.2011.05.020

Zheng, H., M. Li, H. Liu, W. Ren and D.S. Hu *et al*., 2007a. Immunoglobulin alpha heavy chain derived from human epithelial cancer cells promotes the access of s phase and growth of cancer cells. Cell Biol. Int., 31: 82-87. DOI: 10.1016/j.cellbi.2006.09.009

Zheng, H., M. Li, W. Ren, L. Zeng and H.D. Liu *et al*., 2007b. Expression and secretion of immunoglobulin alpha heavy chain with diverse vdj recombinations by human epithelial cancer cells. Molecular Immunol., 44: 2221-2227. DOI: 10.1016/j.molimm.2006.11.010

Zheng, J., J. Huang, Y. Mao, S. Liu and X. Sun *et al*., 2009. Immunoglobulin gene transcripts have distinct vhdjh recombination characteristics in human epithelial cancer cells. J. Biological Chem., 284: 13610-13619. DOI: 10.1074/jbc.M809524200

Zhou, X.X., W.H. Jia, G.P. Shen, H.D. Qin and X.J. Yu *et al*., 2006. Sequence variants in toll-like receptor 10 are associated with nasopharyngeal carcinoma risk. Cancer Epidemiol. Biomarkers Prev., 15: 862-866. DOI: 10.1158/1055-9965.epi-05-0874

Zhu, X., C. Li, X. Sun, Y. Mao and G. Li *et al*., 2008. Immunoglobulin mrna and protein expression in human oral epithelial tumor cells. Applied Immunohistochem. Molecular Morphol., 16: 232-238. DOI: 10.1097/PAI.0b013e31814c915a

Zhu, X., L. Wu, L. Zhang, P. Hao and S. Zhang *et al*., 2010. Distinct regulatory mechanism of immunoglobulin gene transcription in epithelial cancer cells. Cellular Molecular Immunol., 7: 279-286. DOI: 10.1038/cmi.2010.13