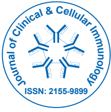
Journal of
Clinical & Cellular Immunology

Lee, J Clin Cell Immunol 2015, 6:6
DOI: 10.4172/2155-9899.1000385

Review Article Open Access

Dual Differential Roles of Cancerous Immunoglobulins as Suggested by Interactions with Human Serum Proteins

Gregory Lee[1,2*]

[1]*UBC Centre for Reproductive Health, Vancouver, Canada*

[2]*Department of Pathology, College of Medicine, Shantou University, Shantou, China*

[*]**Corresponding author:** Gregory Lee, Ph.D., UBC Center for Reproductive Health, 9117 Shaughnessy Street, Vancouver, BC, V6P 6R9, Canada, Tel: 604-325-4602; E-mail: leecyg@gmail.com

Received date: November 16, 2015; **Accepted date:** December 28, 2015; **Published date:** December 31, 2015

Copyright: © 2015 Lee G. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

In search of functional roles of immunoglobulins expressed by cancer cells, molecular interactions between cancerous immunoglobulins and human serum proteins or protein fragments were investigated by using RP215 monoclonal antibody as the unique probe. RP215 was initially generated against OC-3-VGH ovarian cancer cell extract and shown to react with carbohydrate-associated epitope located mainly on the variable regions of immunoglobulin heavy chains and others expressed by cancer cells which are designated in general as CA215. CA215 and cancerous immunoglobulins (cIgG) were affinity isolated from the shed medium of cultured cancer cells. Furthermore, by using purified CA215 and cIgG as the respective affinity ligands, the serum proteins or components were affinity isolated and subject to analysis by LCMS/MS methods. The results of such analysis suggest that as many as 80-86% of the isolated human serum proteins were identical in those purified by either affinity column. They are generally classified as pro-cancer and anti-cancer protein components. Among the known pro-cancer protein components recognized by cancerous immunoglobulins are C4 binding proteins α-chain, complement C3, complement factor H, serotransferrin and vitronectin, etc. On the other hand, inter-α-trypsin inhibitor heavy chain 4, anastellin, apolipoprotein A1, fibrinogen β-chain and keratin type 1 cytoskeletal 9 or autoimmune IgG were considered to be anti-cancer proteins from human serum. Based on these observations, dual functional roles of cancerous immunoglobulins are hypothesized. It has been demonstrated in this study that cancerous immunoglobulins are capable of serving as specific binding protein-like immunoglobulins to capture serum proteins to promote growth of cancer cells. At the same time, they can neutralize those with anti-cancer properties in human circulations.

Keywords: cIgG; CA215; Anti-cancer; Pro-cancer; Immunoglobulin; Human serum protein

Abbreviations

CDC: Complement-dependent Cytotoxicity; cIgG: Cancer cells expressed Immunoglobulin G; IgG: Immunoglobulin G; IgM: Immunoglobulin M; MALDI-TOF MS: Matrix Adsorption Laser Desorption Ionization-Time of Flight Mass Spectrometry; CA215: Cancer-associated antigen recognized by RP215 monoclonal antibody; LC-MS/MS: Liquid Chromatography plus analysis by Mass-Spectrometry; DTT: Dithiothreitol; CA215 S+: Human serum protein captured (adsorbed) by CA215-linked immunoaffinity column; cIgG S +: Human serum proteins captured (adsorbed) by anti-human IgG affinity column

Introduction

Expressions of immunoglobulins in cancer cells were known for decades, even though the mechanisms of action are still not fully understood [1]. This is in contrast to conventional immune system, in which detailed molecular mechanisms of immunoglobulin expressions have been well established [1,2].

Many cancer cells, especially those of the hyperplastic epithelial origins were known to express immunoglobulins of unknown origins [1]. The expressions of immunoglobulins by cancer cells are independent of those by normal B cells [1] and were demonstrated by immunohistochemistry, RT-PCR and IgG-related SiRNA inhibition studies *in vitro* and *in vivo* [2,3]. In addition, anti-human IgG were found to induce apoptosis of almost all cancer cells of different tissue origins *in vitro* [4,5]. Complement-dependent cytotoxicity (CDC) reactions were also demonstrated with anti-human IgG and complement indicating the surface expressions of immunoglobulins [6]. These observations led to a general conclusion that cancer cell-expressed immunoglobulins are essential for the growth/proliferation of cancer cells [7]. Further studies with nude mouse animals also revealed significant reductions in volumes of implanted tumor upon injection of antibodies against human immunoglobulins [8-10]. Additional molecular biological studies also indicated that expressions of cancerous immunoglobulins are quite different for those of immunoglobulins derived from B cells [1]. No class switching and somatic hyper mutation are involved in the V(D)J heavy chain regions of cancer cell-expressed immunoglobulins [11].

To elucidate exact functional roles of cancer cell-expressed immunoglobulins in human environment, interactions between human serum proteins and cancerous immunoglobulins were investigated and analyzed by LCMS/MS and MALDI TOF-MS methods. In 1987, a major breakthrough of this research work came, when a monoclonal antibody designated as RP215 was generated against OC-3-VGH ovarian cancer cell line. It was shown to react specifically with a carbohydrate-associated epitope located mainly on

the variable regions of immunoglobulin heavy chains as well as other types of cancerous glycoproteins designated in general as CA215 [8].

Numerous *in vitro* and *in vivo* immunological and biochemical studies have been performed during the last decade by using RP215 as the unique probe for studies of cancerous immunoglobulins [8,9]. Based on these studies, it was generally concluded that apoptosis can be induced upon incubation of RP215 or antibodies against antigen receptors (including anti-immunoglobulin and anti-T-cell receptors), which was consistent with the hypothesis that cancerous immunoglobulins are essential for growth/proliferation of cancer cells *in vitro*, or *in vivo* [8-10]. Furthermore, *in vivo* nude mouse experiments with implanted tumors from derived cancer cell line revealed that the volume of the implanted tumor was reduced dose-dependently upon treatments with RP215 or antibodies against immunoglobulins [10,11].

Effects of RP215 and antibodies against antigen receptor on the gene regulations of culture cancer cells were also investigated [12]. Changes of a number of genes including those of toll-like receptors and those required for growth/proliferation of cancer cells were observed. As many as ten to twelve genes were observed to change in response to treatments with RP215 or anti-antigen receptors. High correlations (≥ 90%) in gene regulations of RP215 and other two anti-antigen-receptor ligands were observed and demonstrated. Based on the results of gene regulation studies, it was clearly demonstrated that the blockage of cancer immunoglobulin and/or T-cell receptors by RP215 and other anti-antigen receptors can be well demonstrated by inducing apoptosis to cultured cancer cells *in vitro* [13]. Therefore, cancerous immunoglobulins are essential for growth/proliferation of cancer cells *in vitro* and *in vivo*, and affected by RP215 which reacts mainly with cancerous immunoglobulins on the surface of cancer cells. Therefore, RP215 may have potential therapeutic applications as the anti-cancer drug provided that RP215 is humanized for administration in humans [14]. So far, humanized RP215 has been successfully developed and shown to be bioequivalent to murine RP215 and should be ready for applications in preclinical studies [15].

To maintain growth/proliferation of cancer cells in human circulations, interactions with human serum protein components may play essential roles [4]. Therefore, we believe that cancerous immunoglobulins may be involved in such molecular interactions. Therefore, RP215 which reacts mainly and specifically with cancerous immunoglobulins may be utilized as the unique probe for studies of such interactions. Based on this hypothesis, CA215 and cIgG were initially isolated from culture cancer cell lines in shed medium. CA215 and cIgG can be utilized as affinity ligands to capture human serum proteins in separate affinity columns [6,8]. Through sequential affinity chromatographs, the isolated human serum proteins can then be analyzed by LC-MS/MS methods. From the results of such analysis, the functional roles of cancerous immunoglobulins can be revealed and presented in this review [5].

Methods

Affinity-isolation of CA215 and cancerous immunoglobulin as well as human serum protein components

Preliminary studies regarding the affinity isolation of CA215 and cancerous immunoglobulins have been published elsewhere [4,6]. Briefly, CA215 was isolated from shed medium of cultured ovarian OC-3-VGH cancer cells by using RP215-linked affinity column in a typical affinity chromatography. The culture shed medium was first concentrated by adding ammonium sulfate (40% satination) followed by extensive dialysis against PBS, prior to loading on the RP215-linked affinity column. Following extensive wash, the bound CA215 was eluted with 5 mM citrate acid at pH 2.2. After dialysis with PBS, purified CA215 was concentrated and used a ligand to build a CA215-linked immunoaffinity column. Similarly, goat anti-human IgG was utilized as the affinity ligand in an affinity column for purification of cIgG (cancerous immunoglobulins).

To purify human serum protein components which might have affinity or any interactions with CA215 or cIgG were isolated, respectively by CA215- and cIgG linked affinity columns. Following elution, concentration and dialysis in ammonium bicarbonate, purified human protein components which was designated as CA215-S+ and cIgG-S+ were obtained, respectively. They were subjected to analysis by LC-MS/MS methods though a contract service from the University of Victoria Genomic BC Proteomics Centre to be described in the following section.

Analysis of human serum protein components by LC-MS/MS method

Affinity-isolated CA215-S+ or cIgG-S+ (200-500 µg) was rehydrated with a minimum 200 to 500 µl 25 mM ammonium bicarbonate prior to reduction with suitable amount of dithiothreitol (DDT) for 30 min at 37°C in the dark. Alkylation of cysteine sulfhydryl groups was performed with 200 mM DDT and 10 µg trypsin (Promega) was added to each sample for 16 h at 37°C.

A Waters Oasis hydrophilic-lypophilic balanced column (1 ml, 10 mg) was equilibrated with 2 ml acetonitrile and then with 2 ml 0.6% acetic acid. The supernatant was applied to the column by 2 ml 0.6% acetic acid, and peptides eluted with 700 µl 80% acetonitrile plus 0.6% acetic acid. The samples were concentrated *via* speed vac, acidified and desalted with e19 Stage Tips prior to LC-MS/MS analysis.

The peptide mixtures were separated by online reverse-phase chromatography with a Thermo Scientific Easy-Inc. 1000 system at a flow rate of 300 nl/min. the chromatography system was compared online with an Orbitrap Fusion Tribid mass spectrometer (Thermo Scientific, San Josa, CA). The peptide mixture in each sample was subjected to MS analysis by using standard operation manual. Raw files were created by X Caliber 3.0.63 software from Thermo Scientific and analyzed with Proteome Discoverer 1.4.1.1.4 software from the source.

Results and Discussion

Human serum proteins recognized by CA215 and cIgG expressed in cancer cells

The expressions of CA215 and/or cancerous immunoglobulins (cIgG) have been well documented through decades of studies [4,6,8-10,12]. The functional roles of cIgG and/or CA215 can be demonstrated by their interactions with major serum protein components. This can be accomplished by the present study with sequential affinity chromatography by using CA215 or cIgG as the respective affinity ligands [4,6].

Citation: Lee G (2015) Dual Differential Roles of Cancerous Immunoglobulins as Suggested by Interactions with Human Serum Proteins. J Clin Cell Immunol 6: 385. doi:10.4172/2155-9899.1000385

Molecular identity of CA215 expressed in cancer cells

RP215 was initially shown to react specifically with cancer cell-expressed glycoproteins designated in general as CA215 through the binding of a common but unique carbohydrate-associated epitope. The affinity-isolated CA215 was trypsin-digested and subject to analysis by MALDI-TOF MS method [8].

As shown in Table 1, the molecular identity of affinity-isolated CA215 was listed and categorized according to their basic molecular structure, derived from the detected 124 tryptic peptides. From this table, greater than 40% of detected CA215 were found to be immunoglobulins expressed by cancer cells. In addition, a number of immunoglobulin superfamily proteins (IgSF) were also detected, accounting for as many as 60% of the detected protein species. The remaining 35-40% of the detected CA215 are unrelated to IgSF proteins [16].

Molecule function/category	**Number of peptides matched[a,b,c]** **Total=124 (%)**
I. Antigen receptors	
1.Antibodies and immunoglobulins	52 (42.0%)
2.T-cell receptor chains	7 (5.7%)
II. Antigen-presenting molecules (MHC I and MHC II)	6 (4.95)
III. Adhesion molecules	10 (8.1%)
IV. Cytokine and growth factors	8 (6.5%)
V. Receptor tyrosine kinase/phosphatase	7 (5.7%)
VI. Others	
1. IgSF related (e.g., titin)	12 (9.7%) Total with homology; 75/124 (60.5%)
2. IgSF unrelated (e.g., mucin)	9 (7.3%)

[a]Acid –eluted CA215 (lots CA215A and CA215B) was used to MALDI-TOF MS analysis with MASCOT Program from http://www.matrixscience.com.

[b]Excluding overlapping matched peptides.

[c]Reference [16] with permission.

Table 1: Molecular analysis of CA215 based on MALDI-TOF MS analysis of tryptic peptides.

Molecular analysis of human serum proteins designated as CA215 S+ and cIgG S+

Following affinity-isolation of CA215 S+ and cIgG S+ from pooled human serum, many distinct human serum proteins or their components were identified by LC-MS/MS methods. The majority of human serum proteins were commonly detected by both CA215 and cIgG –linked affinity columns (80-86%). They were listed in Table 2 for further investigations regarding anti-cancer or pro-cancer nature of these proteins. The remaining proteins which are recognized either by CA215 or cIgG ligands are listed in Table 3 for comparisons.

Human serum proteins with pro-cancer properties

About 80-86% of the detected human serum protein components were commonly recognized by both CA215 and/or cIgG. Based on the results of previous and ongoing studies by others, part of these proteins were classified to exhibit pro-cancer properties. Further analysis reveals that some are complement-related proteins and serve to prevent complement attack on cancer cells *in vitro* and *in vivo*. Among these are C4b-binding protein α chain, complement C3 and complement factor H.

Proteins or Protein Fragments (Molecular Weight in kDa)	**References**	**Anti-Cancer (A-C) or Pro-Caner (P-C) in nature**
C4b-binding protein α chain (67)	[18,19]	P-C
Complement C3 (187)	[17,18,20]	P-C
Complement factor H (139)	[19,24-26]	P-C
Serotransferrin (77)	[29-31]	P-C
Vitronectin (54)	[35-38]	P-C
Histidine-rich glycoprotein (60)	[48,49]	P-C
α2 macroglobulin (163)	[50,51]	P-C
CD5 antigen-like protein (38)	[52]	P-C
Isoform 2 of α-1-antitrypsin (40)	[53]	P-C
Hemopexin (Beta-1-B glycoprotein) (52)	[54]	P-C
inter-α-trypsin inhibitor heavy chain 4 (104)	[55,56]	A-C
Anastellin (256)	[40,41]	A-C
Apolipoprotein A-1 (31)	[43,44]	A-C
Fibrinogen β chain (56)	[57]	A-C
Keratin type I cytoskeletal 9 (62)	[47]	A-C
Other: Complement component C4b (Childo blood group (193)	[18,20]	A-C

Table 2: List of human serum proteins or protein fragments which are commonly recognized by CA215 and cIgG.

Complement-related components in human serum

1. C4b-binding protein is known to be a soluble complement inhibitor consisting of 7α and one β subunit with a molecular size of 67 kDa [17,18]. By acting as a cofactor, to inhibit complement activation through factor I-mediated degradation of C3b and C4b [19]. This has been demonstrated in SKO-3 and CAOV-3 ovarian adenocarcinoma cell lines, the α-chain of C4b- binding protein is capable of inhibiting the complement classical pathway through the inactivation of C4b. Therefore, it was assumed that interactions of C4b binding protein with C4b-binding proteins in cancer cells would prevent or inhibit complement activation [19]. Therefore, C4b-binding protein α-chain was demonstrated to have pro-cancer function [19].

2. Complement C3 (MWt 187 kDa) and its cleavage C3a and C3b play central roles in the complement cascade and can be engrafted in tumors of mouse animal models to decrease tumor proliferation [17,18]. C3a is the cleavage product of C3 have been found to be associated with activation/proliferation of neoplasma including production of vascular endothelial growth factor (VEGF), extracellular matrix reorganization and disintegration of tumor angiogenesis as well as invasion/migration [20,21]. Complement C3 was also expressed in gastric-cancer related cell lines to promote cancer development and progression [22].

3. Complement factor H is a serum glycoprotein with a molecular weight of 139 kDa, and is key regulator of the alternative pathway in the complement system. Similar to C4b-binding protein, complement factor H acts to prevent complement activation by acting as a cofactor of serine protease factor I to induce cleavage and inactivation of C3b and C4b and to accelerate the degradation of C3 convertase [23]. Complement factor H was found to be expressed in cancer cells of many tissue origins [24-26] rendering them resistant to complement attack and reduce tumor growth. Therefore, it can be hypothesized what complement factor H serves to protect cancer cells from complement activation [24-27].

In summary, the three complement-related proteins or components from human serum were commonly recognized by both CA215 and cIgG expressed by cancer cells. Therefore, certain human serum proteins are important to prevent of complement-inactivation or attack cancer cells in human circulations [27,28].

Serotransferrin and vitronectin

Two human serum components, serotransferrin and vitronectin were also commonly recognized by both CA215 and cIgG [29-36]. Serotransferrin (Serum transferrin) is an iron binding protein of 77 kDa in human serum. It functions to transport iron from degradation to sites of storage and utilization [29-31] and involves in important cellular functions, proliferation in cases of prostate and lung cancer. Therefore, it is believed that blockage of transferrin binding on cancer cell surface will result in tumor growth inhibition and serotransferrin may serve as important growth factor or pro-cancer factor for the proliferation of cancer cells.

Vitronectin is a glycoprotein of 54 kDa produced in liver. Vitronectin is involved in cancer stem cell differentiation of breast and prostate cancer cell. It also interacts with the proteins in the integrin family for the adhesion, differentiation, as well as growth of many cancer cells [35,36].

Therefore, we believe that serotransferrin and vitronectin interacting with cancerous IgG and CA125 are proteins of pro-cancer factors in promoting tumor growth and proliferation [36-38].

Human serum proteins exhibiting anti-cancer properties and interacting with CA215 and cIgG

Anti-cancer properties of several human serum proteins commonly detected by CA215 and cIgG were noticed and presented in this analysis. Most of these human serum proteins are known to exhibit anti-cancer properties through several previous investigations [39-44]. Among these are anastellin, apolipoprotein A1, fibrinogen β-chain and others such as inter-α-trypsin inhibitor and keratin type 1 cytoskeletal 9 [39-45].

1. Anastellin and Apolipoprotein-A1: Anastellin is known to be a fragment of fibrinogen of the first type III module with a molecular size of 256 kDa [39-42] and shown to react with a variety of proteins, including integrins, proteoglycans, fibronectin and fibrinogen. Upon binding with fibronectin, anastellin can promote changes in organization and assembly of fibrinonectin matrix. Anastellin was also found to inhibit tumor growth by inhibiting angiogenesis [39-42] and by blocking cell cycle growth regulations [39-41].

2. Apolipoprotein A-1 is a major protein component of high-density lipoprotein (HDL) with a molecular size of 31 kDa in human plasma [43]. Apolipoprotein A-1 is known to be cardio protective with anti-inflammatory and anti-oxidant properties. It was also considered as a biomarker of ovarian cancer when combined with CA215 to improve diagnostic sensitivity. Apolipoprotein A-1 was also shown to suppress tumor growth, angiogenesis, metastasis in mouse animal model studies [43,44].

3. Fibrinonectin β chain is one of the peptide chains required for the assembly of fibrinogen involved in the formation of blood clot [45,46]. The first 20 amino acid of N terminus of the fibrinogen beta chain (β43-63) significantly inhibit VEGF-activation of epithelial cells to the extracellular matrix, and tumor vascularization and increase tumor necrosis [45]. Therefore, Fibrinonectin β chain which interacts with cIgG or CA215 is considered to exhibit anti-cancer properties in human body [45,46].

4. Anti-cancer nature of two cIgG-recognized human serum proteins were also identified. They are inter-α-trypsin inhibitor heavy chain 4 (35 kDa) [47] and keratin type 1 cytoskeletal 9 (62 kDa) [20]. The former is a fragment of inter-α-trypsin inhibitor heavy chain which was formed to supress proliferation and migration of tumor cell growth. The latter is a structural protein for intermediate filaments in epidermal cells and formed to suppress tumor growth in drug resistant Human breast cancer tissues.

Human serum proteins recognized separately by CA215 and cIgG

As shown in Table 3, several distinct human serum proteins were recognized separately by CA215 and cIgG ligands. This observation may indicate the differences in the molecular nature between CA215 and cIgG. However, a few of these proteins listed in Table 3 are related to pro-cancer or anti-cancer properties to cancer cells [65-74]. The molecular nature and functions of these captured human serum proteins remain to be explored in the future [49-68].

Human serum proteins recognized by CA215 only

As shown in Table 3, certain CA215-recognized human serum proteins exhibit pro-cancer or anti-cancer properties. An investigation on identifying predictors of treatment outcome and survival in patients with non-small cell lung cancer treated with docetaxel has shown that α-1-acid glycoprotein appears to be an independent predictor of response and a major objective prognostic factor: the higher the level of α-1-acid glycoprotein, the shorter the patients survive [60]. Among plasma proteomic analysis of patients, apolipoproteins are among the important proteins with anti-cancer in nature. Apolipoprotein D is a promising prognostic and predictive factor in breast cancer and its determination can be done by immunoelectrophoresis in tumor cytosol or immunohistochemistry [62]. Other proteins exhibiting anti-cancer properties are afamin, apolipoproteins and plasminogen. As a vitamin E binding protein, afamin was found to be a potential

biomarker of ovarian cancer. Although CA-125 is a widely used biomarker for ovarian cancer, its low sensitivity limits diagnosis for early stage patients. Afamin shows potential complementarity with CA-125 in longitudinal monitoring of patients with ovarian cancer [58]. Mainly associated with high-density lipoproteins, apolipoprotein M is identified as a carrier of the bioactive lipid sphingosine-1-phosphate, through which, it affects a wide range of biological processes, such as lymphocyte trafficking, endothelial permeability in the lung, and even virus suppression and cancer [63]. Judged from effects on a syngeneic tumor model in C57/bl6 mice, histidine-rich glycoprotein is demonstrated as a potent inhibitor of tumor vascularization with anticancer properties. A comparison indicated that after treatment with histidine-rich glycoprotein, the growth of fibrosarcoma, a very aggressive tumor, was reduced by >60% [49]. The nature of plasminogen has been investigated both *in vitro* and *in vivo* [67]. The results clearly shown that plasminogen activator inhibitor type 2 acts specifically against prostate cancer cells. The research of its anti-cancer effects on antiproliferation of tumors and prostate cancer lymph node metastasis were carried out. It is proposed that plasminogen could be considered for further development for the therapy of prostate cancer [67].

Recognized by CA215		Recognized by cIgG	
Proteins or protein fragments (molecular weight in kDa)	**References**	**Proteins or protein fragments (molecular weight in kDa)**	**References**
Afamin (69)	A-C[a] [58]	Alpha-1B glycoprotein (54)	U-K[a] [59]
Alpha-1-acid glycoprotein 1 (24)	P-C[a] [60]	Complement C5 (188)	A-C [61]
Apolipoprotein D (21)	P-C [62]	Complement component C9 (63)	A-C [61]
Apolipoprotein M (21)	A-C [63]	Gelsolin (81)	P-C [64]
Apolipoprotein H (38)	U-K [65]	Pregnancy zone protein (164)	U-K [66]
Histidine-rich glycoprotein (60)	A-C [49]	Inter-alpha-trypsin inhibitor heavy chain H2 (106)	A-C [55]
Plasminogen (91)	A-C [67]		
Vitamin D-binding protein (55)	U-K [68]		
[a]A-C: Anti-Cancer; P-C: Pro-Cancer; U-K: Unknown			

Table 3: Human serum proteins fragments recognized by either CA215 or cIgG.

Human serum proteins recognized by cIgG only

On the right column of Table 3, gelsolin exhibits pro-cancer properties; but Inter-alpha-trypsin inhibitor heavy chain H2 and complements exhibits anti-cancer properties. Tumor gelsolin is associated with protein overexpression of breast cancer patients. Although gelsolin alone is not a prognostic factor, gelsolin coexpression may be an important additional prognostic factor in some breast cancer patients [64]. As a central part of the innate immune system, complements such as complement 3, 5, and 9 provides a highly effective means for destruction of invading microorganisms and elimination of dead and apoptotic cells [61]. While the complements are activated, they can suppress the growth of cancer cells. However, cancer cells seem to be able to establish a convenient balance between complement activation and inhibition. Some attempts have been already made to modulate antibody-mediated complement activation to increase the complement-fixing capacity of the therapeutic antibodies. With the introduction of monoclonal antibodies in cancer immunotherapy, complement has come into play a great potential as anticancer proteins [61]. The study on inter-alpha-trypsin inhibitor heavy chains molecules has been shown to play a particularly important role in inflammation and carcinogenesis [55]. Inter-alpha-trypsin inhibitor heavy chain genes are clearly downregulated in multiple human solid tumors, including breast, colon and lung cancer. The results indicate that inter-alpha-trypsin inhibitor heavy chains may represent a family of putative tumor suppressor or anti-cancer proteins [55].

Potential application of RP215 in cancer immunotherapy

Expressions of surface immunoglobulins in cancer cells are almost universal with few exceptions and can be targeted for cancer immunotherapy [65,70]. Since the discovery in 1987, RP215 has been used as a unique probe for studies of immunoglobulins expressed on the surface of cancer cells [10]. Biological and immunological studies *in vitro* during the last decade indicated that RP215 can serve as the substitute for anti-immunoglobulins to target this surface bound molecules to induce apoptosis and complement-dependent cytotoxicity reactions to cancer cells [71]. *In vitro* nude mouse experiments also suggested that volumes of implanted tumors where reduced significantly in model studies [12,71-73]. Therefore, RP215 has been humanized to make it suitable for human applications in immunotherapy of almost all human cancer following appropriate preclinical and clinical studies [74] in the near future.

Conclusions

In this study, quite a few human serum proteins were shown to interact with both cIgG and CA215 and exhibit either pro-cancer or anti-cancer properties. As many as 80-86% of the affinity isolated human serum proteins were commonly captured by both CA215 and cIgG affinity ligands and identified by LC-MS/MS methods. This is not totally unexpected since the majority of identified CA215 glycoproteins are found to be cancerous immunoglobulins (≥ 40-60%) (Table 1).

From this study, the functional roles of cIgG were clearly demonstrated. CA215 and cIgG were found to recognize a number of human serum proteins which exhibit either pro-cancer or anti-cancer properties in nature (Table 2). Therefore, the surface bound cIgG in cancer cells are capable of interacting with human serum protein components, which can be isolated by affinity chromatography. On the other hand, serum proteins with pro-cancer properties were captured by cIgG to promote the growth/proliferation of cancer cells in circulation. At the same time, serum proteins with anti-cancer properties can also be neutralized by cIgG or CA215 on the surface of cancer cells to minimize damages to cancer cells. In view of the fact that CA215 consist mainly of heavy chains of cIgG, both exhibit similar functional roles in cancer cells. In previous studies [9], both were found to induce apoptosis and complement-dependent cytotoxity to cancer cells upon binding with antibodies against cIgG or RP215 which reacts specifically with CA215 or cIgG [4,6].

The exact mode of interactions between CA215 or cIgG and human serum proteins are currently unknown. It could be simply antigen-antibody bindings. It may also be receptor-ligand-mediated interactions. This remains to be further investigated with each specific case.

Therefore, in this study, we have shown that cIgG, cancer-cell-expressed immunoglobulins and CA215 were able to recognize human serum proteins, some of which were shown to exhibit pro-cancer or anti-cancer properties. Therefore, dual functional roles of cIgG in cancer cells can be clearly demonstrated through their mutual interactions with human serum proteins. These observations may help to explain why cancer cells are able to survive and proliferate under our natural human environment with the widespread expressions of surface-bound cIgG.

References

1. Qiu X, Zhu X, Zhang L, Mao Y, Zhang J, 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.
2. Murphy K, Travers P, Walport H (2008) The Adaptive Immune Response. In: Janeway's Immunology. (7thedn), Garland Science, Taylor France's Group, pp. 323-496.
3. Cao Y, Sun Y, Poirier S, Winterstein D, Hegamyer G, et al. (1991) Isolation and partial characterization of a transformation-associated sequence from human nasopharyngeal carcinoma. Mol Carcinog 4: 297-307.
4. Lee G, Huang CY, Liu S, Zhang H (2013) The Immunology of Cancer Cells. Symbiosis Open J Immunol 1: 4-7.
5. Lee G (2015) Functional Roles of Cancerous Immunoglobulins and Potential Applications in Cancer Immunodiagnostics and Immunotherapy. In: Khan WA (ed) Innovative Immunology. Austin Publishing Group, New Jersey.
6. Lee G, Huang CY, Tang Y, Zhang H (2013) Potential Roles of Cancerous Immunoglobulins in the Immunology of Cancer Cells. J Clin Cell Immunol 5: 200-206.
7. 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.
8. Lee G, Laflamme E, Chien CH, Ting HH (2008) Molecular identity of a pan cancer marker, CA215. Cancer Biol Ther 7: 2007-2014.
9. Lee G, Ge B (2010) Inhibition of in vitro tumor cell growth by RP215 monoclonal antibody and antibodies raised against its anti-idiotype antibodies. Cancer Immunol Immunother 59: 1347-1356.
10. Lee G, Chu RA, Ting HH (2009) Preclinical assessment of anti-cancer drugs by using RP215 monoclonal antibody. Cancer Biol Ther 8: 161-166.
11. Huang J, Sun X, Mao Y, Zhu X, Zhang P, 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.
12. Tang Y, Zhang H, Lee G (2013) Similar Gene Regulation Patterns for Growth Inhibition of Cancer Cells by RP215 or Anti-Antigen Receptors. J Cancer Sci Ther 5: 200-208.
13. Lee G, Zhu M, Ge B, Potzold S (2012) Widespread expressions of immunoglobulin superfamily proteins in cancer cells. Cancer Immunol Immunother 61: 89-99.
14. Lee G, Cheung AP, Ge B, Zhu M, Giolma B, et al. (2012) CA215 and GnRH receptor as targets for cancer therapy. Cancer Immunol Immunother 61: 1805-1817.
15. Lee G, Huang CY, Ge BX (2014) Two Distinct Humanized Monoclonal Antibodies for Immunotherapy of Ovarian Cancer. J Cancer Sci Ther 6: 110-116.
16. Lee G, Azadi P (2012) Peptide Mapping And Glycoanalysis of Cancer Cell–Expressed Glycoproteins CA215 Recognized by RP215 Monoclonal Antibody. J Carbohydrate Chem 31: 10-30.
17. Markiewski MM, DeAngelis RA, Benencia F, Ricklin-Lichtsteiner SK, Koutoulaki A, et al. (2008) Modulation of the antitumor immune response by complement. Nat Immunol 9: 1225-1235.
18. Markiewski MM, Lambris JD (2009) Is complement good or bad for cancer patients? A new perspective on an old dilemma. Trends Immunol 30: 286-292.
19. Holmberg MT, Holmberg MT, Blom AM, Meri S (2001) Regulation of Complement Classical Pathway by Association of C4b-Binding Protein to the Surfaces of SK-OV-3 And CAOV-3 Ovarian Adenocarcinoma Cells. J Immunol 167: 935-939.
20. Rutkowski MJ, Sughrue ME, Kane AJ, Mills SA, Parsa AT (2010) Cancer and the complement cascade. Mol Cancer Res 8: 1453-1465.
21. Vivanco I, Sawyers CL (2002) The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer 2: 489-501.
22. Kitano E, Kitamura H (1993) Synthesis of the third component of complement (C3) by human gastric cancer-derived cell lines. Clin Exp Immunol 94: 273-278.
23. Józsi M, Zipfel PF (2008) Factor H family proteins and human diseases. Trends Immunol 29: 380-387.
24. Ajona D, Castaño Z, Garayoa M, Zudaire E, Pajares MJ, et al. (2004) Expression of complement factor H by lung cancer cells: effects on the activation of the alternative pathway of complement. Cancer Res 64: 6310-6318.
25. Junnikkala S, Jokiranta TS, Friese MA, Jarva H, Zipfel PF, et al. (2000) Exceptional resistance of human H2 glioblastoma cells to complement-mediated killing by expression and utilization of factor H and factor H-like protein 1. J Immunol 164: 6075-6081.
26. Junnikkala S, Hakulinen J, Jarva H, Manuelian T, Bjørge L, et al. (2002) Secretion of soluble complement inhibitors factor H and factor H-like protein (FHL-1) by ovarian tumour cells. Br J Cancer 87: 1119-1127.
27. Wilczek E, Rzepko R, Nowis D, Legat M, Golab J, et al. (2008) The possible role of factor H in colon cancer resistance to complement attack. Int J Cancer 122: 2030-2037.
28. Ajona D, Hsu YF, Corrales L, Montuenga LM, Pio R (2007) Down-regulation of human complement factor H sensitizes non-small cell lung cancer cells to complement attack and reduces in vivo tumor growth. J Immunol 178: 5991-5998.
29. Ponka P (1999) Cellular Iron Metabolism. Kidney Int 55: S2-S11.
30. Schaeffer E, Boissier F, Py MC, Cohen GN, Zakin MM (1989) Cell type-specific expression of the human transferrin gene. Role of promoter, negative, and enhancer elements. J Biol Chem 264: 7153-7160.
31. Laskey J, Webb I, Schulman HM, Ponka P (1988) Evidence that transferrin supports cell proliferation by supplying iron for DNA synthesis. Exp Cell Res 176: 87-95.
32. Rossi MC, Zetter BR (1992) Selective stimulation of prostatic carcinoma cell proliferation by transferrin. Proc Natl Acad Sci U S A 89: 6197-6201.
33. Vostrejs M, Moran PL, Seligman PA (1988) Transferrin synthesis by small cell lung cancer cells acts as an autocrine regulator of cellular proliferation. J Clin Invest 82: 331-339.
34. Trowbridge IS, Lopez F (1982) Monoclonal Antibody to Transferrin Receptor Blocks Transferrin Binding and Inhibits Human Tumor Cell Growth In Vitro. Proc Natl Acad Sci U S A 79: 1175-1179.
35. Felding-Habermann B, Cheresh DA (1993) Vitronectin and its receptors. Curr Opin Cell Biol 5: 864-868.
36. Hurt EM, Chan K, Serrat MA, Thomas SB, Veenstra TD, et al. (2010) Identification of vitronectin as an extrinsic inducer of cancer stem cell differentiation and tumor formation. Stem Cells 28: 390-398.
37. Kenny HA, Kaur S, Coussens LM, Lengyel E (2008) The initial steps of ovarian cancer cell metastasis are mediated by MMP-2 cleavage of vitronectin and fibronectin. J Clin Invest 118: 1367-1379.
38. Pirazzoli V, Ferraris GM, Sidenius N (2013) Direct evidence of the importance of vitronectin and its interaction with the urokinase receptor in tumor growth. Blood 121: 2316-2323.

39. Ambesi A, McKeown-Longo PJ (2009) Anastellin, the Angiostatic Fibronectin Peptide, is a Selective Inhibitor of Lysophospholipid Signaling. Mol Cancer Res 7: 255-265.
40. Yi M, Ruoslahti E (2001) A fibronectin fragment inhibits tumor growth, angiogenesis, and metastasis. Proc Natl Acad Sci U S A 98: 620-624.
41. Neskey DM, Ambesi A, Pumiglia KM, McKeown-Longo PJ (2008) Endostatin and anastellin inhibit distinct aspects of the angiogenic process. J Exp Clin Cancer Res 27: 61.
42. Pasqualini R, Bourdoulous S, Koivunen E, Woods VL Jr, Ruoslahti E (1996) A polymeric form of fibronectin has antimetastatic effects against multiple tumor types. Nat Med 2: 1197-1203.
43. Zamanian-Daryoush M, Lindner D, Tallant TC, Wang Z, Buffa J, et al. (2013) The cardioprotective protein apolipoprotein A1 promotes potent anti-tumorigenic effects. J Biol Chem 288: 21237-21252.
44. Macuks R, Baidekalna I, Gritcina J, Avdejeva A, Donina S (2010) Apolipoprotein A1 and Transferrin as Biomarkers in Ovarian Cancer Diagnostics. Acta Chirurgica Latviensis 10: 16-20.
45. Zacharski LR, Memoli VA, Rousseau SM (1986) Coagulation-cancer interaction in situ in renal cell carcinoma. Blood 68: 394-399.
46. Krajewska E, Lewis CE, Chen YY, Welford A, Tazzyman S, et al. (2010) A Novel Fragment Derived from the [Beta] Chain of Human Fibrinogen, [Beta] 43-63, is a Potent Inhibitor of Activated Endothelial Cells In Vitro and In Vivo. Br J Cancer 102: 594-601.
47. Yi W, Peng J, Zhang Y, Fu F, Zou Q, et al. (2013) Differential Protein Expressions in Breast Cancer between Drug Sensitive Tissues and Drug Resistant Tissues. Zhong Nan Da Xue Xue Bao Yi Xue Ban 38: 148-154.
48. Jones AL, Hulett MD, Parish CR (2005) Histidine-rich glycoprotein: A novel adaptor protein in plasma that modulates the immune, vascular and coagulation systems. Immunol Cell Biol 83: 106-118.
49. Olsson AK, Larsson H, Dixelius J, Johansson I, Lee C, et al. (2004) A fragment of histidine-rich glycoprotein is a potent inhibitor of tumor vascularization. Cancer Res 64: 599-605.
50. Smorenburg SM, Griffini P, Tiggelman AB, Moorman AF, Boers W, et al. (1996) alpha2-Macroglobulin is mainly produced by cancer cells and not by hepatocytes in rats with colon carcinoma metastases in liver. Hepatology 23: 560-570.
51. Misra UK, SV Pizzo (2012) Upregulation of mTORC2 activation by the selective agonist of EPAC, 8-CPT-2Me-cAMP, in prostate cancer cells: assembly of a multiprotein signaling complex. J Cell Biochem 113: 1488-1500.
52. Li Y, Qu P, Wu L, Li B, Du H, et al. (2011) Api6/AIM/SpÎ±/CD5L overexpression in alveolar type II epithelial cells induces spontaneous lung adenocarcinoma. Cancer Res 71: 5488-5499.
53. López-Árias E, Aguilar-Lemarroy A, Felipe Jave-Suárez L, Morgan-Villela G, Mariscal-Ramírez I, et al. (2012) Alpha 1-antitrypsin: a novel tumor-associated antigen identified in patients with early-stage breast cancer. Electrophoresis 33: 2130-2137.
54. Nakajima S, Moriyama T, Hayashi H, Sakata I, Nakae Y, et al. (2000) Hemopexin as a carrier protein of tumor-localizing Ga-metalloporphyrin-ATN-2. Cancer Lett 149: 221-226.
55. Hamm A, Veeck J, Bektas N, Wild PJ, Hartmann A, et al. (2008) Frequent expression loss of Inter-alpha-trypsin inhibitor heavy chain (ITIH) genes in multiple human solid tumors: a systematic expression analysis. BMC Cancer 8: 25.
56. Huang M, Wang Q, Zhang W, Li DR, Li L (2013) [Impact of the biological function on epithelial ovarian cancer with ITIH4 gene expression down-regulating in vitro]. Zhonghua Fu Chan Ke Za Zhi 48: 34-40.
57. Krajewska E, Lewis CE, Chen YY, Welford A, Tazzyman S, et al. (2010) A novel fragment derived from the beta chain of human fibrinogen, beta43-63, is a potent inhibitor of activated endothelial cells in vitro and in vivo. Br J Cancer 102: 594-601.
58. Jackson D, Craven RA, Hutson RC, Graze I, Lueth P, et al. (2007) Proteomic profiling identifies afamin as a potential biomarker for ovarian cancer. Clin Cancer Res 13: 7370-7379.
59. Jeong DH, Kim HK, Prince AE, Lee DS, Kim YN, et al. (2008) Plasma proteomic analysis of patients with squamous cell carcinoma of the uterine cervix. J Gynecol Oncol 19: 173-180.
60. Bruno R, Olivares R, Berille J, Chaikin P, Vivier N, et al. (2006) Alpha-1-acid glycoprotein as an independent predictor for treatment effects and a prognostic factor of survival in patients with non-small cell lung cancer treated with docetaxel. Clin Cancer Res 9: 1077-1082.
61. Pio R (2006) Control of complement activation by cancer cells and its implications in antibody-mediated cancer immunotherapy. Inmunología 25: 173-187.
62. Søiland H, Skaland I, Janssen EA, Gudlaugsson E, Körner H, et al. (2008) Comparison of apolipoprotein D determination methods in breast cancer. Anticancer Res 28: 1151-1160.
63. Arkensteijn BW, Berbée JF, Rensen PC, Nielsen LB, Christoffersen C (2013) The apolipoprotein m-sphingosine-1-phosphate axis: biological relevance in lipoprotein metabolism, lipid disorders and atherosclerosis. Int J Mol Sci 14: 4419-4431.
64. Thor AD, Edgerton SM, Liu S, Moore DH 2nd, Kwiatkowski DJ (2001) Gelsolin as a negative prognostic factor and effector of motility in erbB-2-positive epidermal growth factor receptor-positive breast cancers. Clin Cancer Res 7: 2415-2424.
65. McNeil HP, Simpson RJ, Chesterman CN, Krilis SA (1990) Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: beta 2-glycoprotein I (apolipoprotein H). Proc Natl Acad Sci U S A 87: 4120-4124.
66. Damber MG, von Schoultz B, Stigbrand T (1976) On the occurrence of the pregnancy zone protein (PZ) in gynecological cancer. Arch Gynakol 221: 97-101.
67. Li Y, Rizvi SM, Ranson M, Allen BJ (2002) 213Bi-PAI2 conjugate selectively induces apoptosis in PC3 metastatic prostate cancer cell line and shows anti-cancer activity in a xenograft animal model. Br J Cancer 86: 1197-1203.
68. Ying HQ, Sun HL, He BS, Pan YQ, Wang F, et al. (2015) Circulating vitamin D binding protein, total, free and bioavailable 25-hydroxyvitamin D and risk of colorectal cancer. Sci Rep 5: 7956.
69. Lee G (2014) Cancerous immunoglobulins in cancer immunology. J Clin Cell Immunol 5: 279.
70. Lee G, Huang CY, Zhang H, Tang Y (2014) The Relationships between Toll-like Receptors and RP215-associated Immunoglobulins Expressed by Cancer Cells. J Cancer Sci Ther 6: 077-080.
71. Lee G, Ge B (2010) Inhibition of in vitro tumor cell growth by RP215 monoclonal antibody and antibodies raised against its anti-idiotype antibodies. Cancer Immunol Immunother 59: 1347-1356.
72. Giavazzi R, Jessup JM, Campbell DE, Walker SM, Fidler IJ (1986) Experimental nude mouse model of human colorectal cancer liver metastases. J Natl Cancer Inst 77: 1303-1308.
73. Liu X, Liu J, Guan Y, Li H, Huang L, et al. (2012) Establishment of an orthotopic lung cancer model in nude mice and its evaluation by spiral CT. J Thorac Dis 4: 141-145.
74. Liu Y, Liu D, Wang C, Liao Q, Huang J, et al. (2015) Binding of the monoclonal antibody RP215 to immunoglobulin G in metastatic lung adenocarcinomas is correlated with poor prognosis. Histopathology 67: 645-653.