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Cancerous Immunoglobulins in Cancer Immunology

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Abstract

The expression of immunoglobulins by cancer cells have been known for two decades. However, the mechanisms of action behind these cancerous immunoglobulins (cIgG) are not well understood and may be different from immunoglobulins secreted by normal B lymphocytes. Therefore, the structural and functional roles of cIgG have been actively investigated to resolve this mystery. A monoclonal antibody, RP215, was generated in 1987 and was shown to react with a carbohydrate-associated epitope localized mainly on the heavy chains of cIgG but not on normal immunoglobulins. The knowledge of cIgG has been greatly advanced by using RP215 as a unique probe. Not only was RP215 shown to act as an anti-cIgG to induce apoptosis of cultured cancer cells *in vitro/in vivo*, it was also shown to elicit complement-dependent cytotoxicity (CDC) reactions to trigger lysis of cancer cells. It has now been established that besides serving to preserve growth/proliferation of cancer cells by capturing growth factors from the human serum, cIgG may also interact with certain human serum proteins which may be harmful to cancer cells. Thus, the hypothesis of dual functional roles of cIgG can be adequately explained in cancer immunology. Furthermore, RP215 may also be used to target cancer cells with surface bound cIgG for development of antibody-based anti-cancer drugs, provided that bioequivalent humanized RP215 is available for preclinical and clinical studies of immunotherapy.

Abbreviations

ALP: Alkaline Phosphatase; cIgG: Cancerous Immunoglobulins; CDC: Complement-Dependent Cytotoxicity; HRP: Horseradish Peroxidase; IHC: Immunohistochemical; IgSF: Immunoglobulin Superfamily; LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry; MALDI-TOF MS: Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry; Mab: Monoclonal Antibody; NFκB: Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; TCRs: T Cell Receptors; TLRs: Toll-Like Receptors.

Background Information

Although the expressions of immunoglobulins by cancer cells have been known for decades, their potential functional roles in the immunology of cancer cells are not fully recognized [1-17]. In comparison, in conventional immunology, the mechanisms of action behind immunoglobulins secreted by B cells have been fully elucidated for decades [18].

As early as 1987, a Mab designated as RP215 was generated against the OC-3-VGH ovarian cancer cell line, and later shown by MALDI-TOF MS to recognize a carbohydrate-associated epitope located mainly on the heavy chains of immunoglobulins expressed by almost all cancer cells [8,19-21]. Since then, RP215 has become a unique tool for investigations regarding the functional roles of cancerous immunoglobulins (cIgG) in cancer cells [20,22-27]. During the last decade, extensive biological and immunological studies have been conducted by use of this antibody probe. Results of such studies revealed dual functional roles of cIgG for the growth/proliferation and the immune protection of cancer cells in our body's microenvironment. Therefore, in this review, our research efforts, as well as that of others on subjects related to cIgG, were summarized to explore the potential roles of cIgG and their implications in cancer immunology and immunotherapy.

Discovery of Immunoglobulins Expressed By Cancer Cells

In conventional immunology, immunoglobulins of unlimited diversity were known to be secreted by B lymphocytes in response to challenge by antigens, such as foreign pathogens or antigen fragments, upon interactions with antigen-presenting cells. The initial interactions are followed by B cell activation, serial differentiations and diversifications of immunoglobulins through processes of somatic hypermutation, and class switching [18,28].

However, immunoglobulins can also be expressed by and detected on the cancer cell surface or in shed culture media [1,7,9-11,14,16,29-35]. These atypical immunoglobulins can also be expressed in normal hyperplastic epithelial cells, as well as those localized in the immune-privileged sites such as the skin, cervix, esophagus, eye, and placenta [2,5,6,29,36-39]. Control experiments were performed to rule out possible contaminations by the endogenous B cells, such as the absence of the CD19 biomarker [29,31,40]. Single cell RT-PCR was eventually utilized to document their expressions from cancer cells of various tissue origins [1,2,7,8,11].

Following the generation of RP215 Mab and identification of the molecular identity of the cognate antigen, designated as CA215, it has become apparent that RP215 reacts specifically with a carbohydrate-associated epitope located mainly on the heavy chains of immunoglobulins expressed by cancer cells [19,41]. Therefore, the existence of this RP215-specific epitope makes cIgG distinguishable from normal human IgG. RP215 has been an invaluable tool to study cIgG, especially their functional roles in cancer cells. The results and conclusions of such studies will be described and summarized in the latter sections of this review.

Following the initial discoveries of cIgG, extensive biological and immunological studies have been performed by several other laboratories [2,11,13,24,32]. Immunohistochemical studies revealed that immunoglobulins can be expressed by almost all cancerous tissues or cancer cell lines [20]. The functional roles of cIgG were initially investigated with anti-human IgG as the probe. It was generally observed that anti-human IgG can induce apoptosis in cancer cell culture and reduce tumor volume in nude mouse animal models [2,11,13,24,32]. These results are consistent with those of siRNA experiments which revealed inhibition of tumor growth *in vitro* or *in vivo* [11,32]. Therefore, it was generally believed that immunoglobulins expressed by cancer cells are essential for the growth/proliferation of cancer cells *in vitro* or *in vivo*. Consistently, when RP215 was incubated with cultured cancer cells for 24 or 48 hrs, apoptosis was induced to cultured cancer cells at a concentration as low as 1μg/mL. Therefore, RP215 can be a suitable substitute for anti-human IgG for many functional studies of cIgG [20,22-27]. Furthermore, RP215 has little cross-reactivity with normal immunoglobulins [42]. Therefore, surface-bound cIgG can be a potential target of RP215 immunotherapy of human cancer if humanized forms of RP215 are available for further clinical studies.

Comparative Structural and Molecular Biological Studies between Cancerous and Normal Immunoglobulins

Since the initial observation of the expression of immunoglobulin among cancer cells, numerous reports have been available to document their expressions through biological and immunological studies, including IHC assays, RT-PCR, *in situ* hybridization, and Western blot assays [20,41,42]. Generally speaking, at the DNA and protein levels in the heavy chain constant regions, immunoglobulins derived from normal B cells or cancer cells are virtually identical [15]. In contrast to a much greater diversity for the heavy chain variable regions of B cell-derived immunoglobulins, the corresponding variable regions of cIgG were found to be much more restricted in hypermutations with no class switching observed [43,44]. The promoter used for the gene expressions of cIgG was also different from that of B cells (Oct-2 vs. Oct-1) [15,45]. Therefore, it was hypothesized that the cancer cell-derived immunoglobulin gene may have a distinct repertoire and may play a role in carcinogenesis [15]. Following the elucidation of the molecular identity of CA215, the cognate antigen for RP215, it has been documented that RP215 reacts specifically with the carbohydrate-associated epitope located mainly on the heavy chains of immunoglobulins expressed by cancer cells but not in normal immunoglobulins. The structure of the RP215-specific "sugar" epitope of cIgG was then elucidated through comprehensive glycoanalysis of CA215 or cIgG to be described later.

Carbohydrate-Associated Epitope Recognized by RP215 Monoclonal Antibody

Since the RP215-specific epitope was sensitive to treatment of mild periodate solution, it was initially concluded that a carbohydrate is involved in the epitope recognition by RP215 [22,41]. Upon treatment of cultured cancer cells with tunicamycin, which inhibits the biosynthesis of N-glycans, the RP215-specific epitope was not affected [46]. Therefore, we believe that the RP215-specific sugar epitope is O-linked, but not N-linked.

Furthermore, the immunoactivity of CA215 was strongly inhibited by goat anti-human IgG (Fab) but not by goat anti-human IgG (Fc). This observation seems to indicate that the O-linked glycan recognized by RP215 is located in the variable regions of the immunoglobulin heavy chains and not in the constant region (Fc) of immunoglobulins. Peptide mapping and glycoanalysis of cIgG or CA215 were performed to elucidate the O-glycan structure that can be recognized by RP215 [46]. The results seemed to indicate that the O-linked glycans from CA215 and cIgG contain trisaccharides with a terminal N-acetyl neuramic acid (NeuAc) as the major glycan component. The glycosyl linkage indicates that the main O-glycan is core I structures with 3-linked and 3,6-linked GalNAcitol (Figure 1) [46]. Therefore, both CA215 and cIgG were found to have identical O-linked structures. Since the affinity between the "sugar" epitope and RP215 is exceptionally high (≤1 nM) for any carbohydrate-related epitope, additional amino acid residues may be involved in the coordinated binding to RP215 for the epitope integrity. This assumption remains to be proven by further in-depth glycoanalysis and glycopeptide mapping [46].
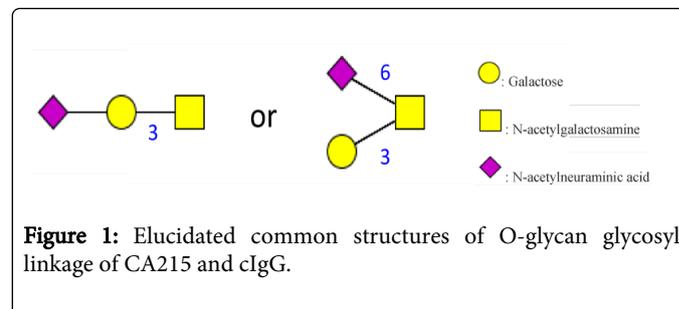
Figure 1: Elucidated common structures of O-glycan glycosyl linkage of CA215 and cIgG.

Widespread Expressions of T cell Receptors and Immunoglobulin Superfamily Proteins in Cancer Cells

During the course of MALDI-TOF MS analysis of CA215, it was noticed that tryptic fragments of T cell receptors were identified in addition to cIgG and many other unidentified IgSF proteins [20,42]. Biological and immunological studies were conducted to demonstrate that both α and β subunits of TCRs are expressed on the surface of more than 80% of cancer cells [20]. At the DNA and protein levels, cancerous TCRs are identical to those in normal T cells. By IHC and Western blot assays, expressions of TCRs in cancer cells can be ascertained. Furthermore, antibodies against TCRs were shown to induce apoptosis and CDC to cancer cells, indicating the surface nature of TCRs on the cancer cell surface. The gene expressions of TCRs among cancer cells were initially documented in 1998, through RT-PCR of single cancer cells [7]. It was not realized until a decade later that the expression of TCRs are widespread among most cancer cells of various tissue origins [20]. At the same time, co-receptors or co-stimulators of TCRs, including CD3, CD4, and CD8 were not

found to be as significantly expressed compared to the high expression level of TCRs [20]. Therefore, TCRs on cancer cells may be different from those on normal T lymphocytes in terms of their mechanisms of action [20]. It remains to be demonstrated whether TCRs on the cancer cell surface serve to recognize any pathogenic peptides or fragments from any cells bearing the major histocompatibility complex (MHC) inside the human body.

Functional Roles of Cancerous Immunoglobulins

Cancerous immunoglobulins were found to be essential for the growth/proliferation of cancer cells. This conclusion was mainly based on the fact that IgG-specific siRNA or anti-human IgG inhibit the growth of cancer cells *in vitro* or *in vivo* with nude mouse animal models [20,22-27]. Similarly, RP215, which reacts specifically with the "sugar" epitope of cIgG was also shown to induce apoptosis among various cancer cells in culture. In addition, CDC reactions can also be triggered in the presence of complement and anti-human IgG or RP215. Furthermore, as one of the surface-bound antigen receptors, antibodies against TCRs can also induce apoptosis to culture cancer cells and CDC reactions in the presence of complement [47].

Gene Regulation Studies of Antigen Receptors Expressed By Cancer Cells

By using semi-quantitative RT-PCR, changes in the regulations of a number of genes were studied in response to treatments of culture cancer cells with antibodies against antigen receptors, notably, anti-human IgG, RP215, and anti-TCR. Since TLRs are critical components for the innate immunity of many cancer cells, the gene regulations of these TLR genes were extensively studied with unrelated cancer cell lines, namely the OC-3-VGH (ovarian) and C-33A (cervical), which were employed as the models [47,48].

Generally speaking, in response to the treatments of antibodies against antigen receptors or RP215 in cancer cells, the NFκB-1 gene was significantly upregulated in both cancer cell lines. Similarly, the TLR-3 gene was also upregulated. In contrast, the genes of TLR-4 and TLR-9 were downregulated significantly [47]. Results of these gene regulation studies seemed to suggest that antigen receptors play important roles to the innate immunity of cancer cells. In addition, when regulations of more than a dozen genes were compared for correlation studies between RP215 and other two antigen receptors, results of high correlations were obtained ($R^2 \geq 0.90$-0.95).

RP215-Specific Epitope in the Diagnosis of Cancer Metastasis and Immunodiagnostic Applications

Since the RP215-specific epitope is carbohydrate-associated, the degree of glycosylations in cIgG may be relevant to the metastasis of cancer cells. By using IHC, flow cytometry, and/or indirect immunofluorescent assays, RP215 can be utilized as a diagnostic tool in determining proliferation, migration, and metastasis of cancer cells in cancerous tissues and in identifying cancer stem cells [49].

Since CA215 was shown to consist mainly of immunoglobulin heavy chains expressed by cancer cells, both cIgG and CA215 can be affinity-isolated and quantitatively determined. CA215 and cIgG can also be assayed in human serum specimens through appropriate enzyme immunoassay kits. RP215 and/or anti-human IgG can be used either for capturing or signal detections. Results of such comparative

assays for CA215, cIgG, and normal human IgG are presented as histograms in Figure 2 [42].
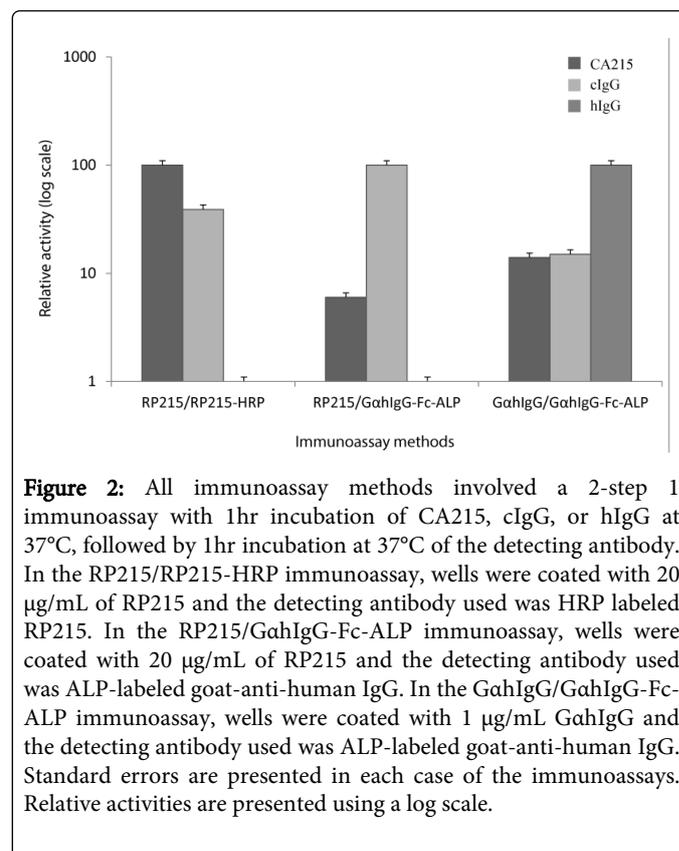
Figure 2: All immunoassay methods involved a 2-step 1 immunoassay with 1hr incubation of CA215, cIgG, or hIgG at 37°C, followed by 1hr incubation at 37°C of the detecting antibody. In the RP215/RP215-HRP immunoassay, wells were coated with 20 μg/mL of RP215 and the detecting antibody used was HRP labeled RP215. In the RP215/GαhIgG-Fc-ALP immunoassay, wells were coated with 20 μg/mL of RP215 and the detecting antibody used was ALP-labeled goat-anti-human IgG. In the GαhIgG/GαhIgG-Fc-ALP immunoassay, wells were coated with 1 μg/mL GαhIgG and the detecting antibody used was ALP-labeled goat-anti-human IgG. Standard errors are presented in each case of the immunoassays. Relative activities are presented using a log scale.

Generally speaking, CA215 and cIgG have relatively low immunoactivity when an anti-hIgG/anti-hIgG-ALP EIA system was used to compare with that of human IgG. However, under a RP215/RP215-HRP or RP215/anti-human IgG-Fc-ALP system, no immunoactivity with human IgG was observed while differential activities of CA215 and cIgG were observed. When the enzyme immunoassay was formulated based on the use of the RP215/RP215-HRP system, signal detection of CA215 in human serum specimens was confirmed in patients with different cancers [21]. When the assay results of other known cancer biomarkers are combined with those of CA215 immunoassays, it was generally concluded that positive detection rates of each cancer category were higher than those using single markers alone. For example, alfafetoprotein (AFP) and CA215 can be combined for a better diagnosis of hepatoma [50]. Similarly, the combined use of CA215 and CA125 can significantly improve the detection of ovarian cancer [21,50]. Results of such analyses are presented in Table 1 for different types of cancer in humans.

CA215 and cIgG can also be readily detected by Western blot assay with RP215 as the probe in human serum specimens obtained at the advanced stages of many cancers [8,25,42,51]. These cancer patient specimens included those of the esophagus, stomach, breast, colon, and liver. When RP215 was used, cIgG from those patient serum specimens can be detected in the Western blot assay based on the 55 kDa molecular weight of IgG heavy chains [41,42]. Thus, it can be confirmed that CA215 consists mainly of cIgG which can be recognized by RP215, whereas normal human IgG shows an absence of cross-reactivity to RP215 [24].

Dual Roles of Cancerous Immunoglobulins in Cancer Immunology

Since the initial observations of immunoglobulins expressed by cancer cells, it was soon demonstrated that cIgG are crucial to the growth/proliferation of cancer cells *in vitro* and *in vivo* [10,11,32]. Following the generation of RP215 which specifically recognizes the "sugar" epitope attached to mainly the heavy chains of cIgG, it has become possible to utilize this unique probe to explore all possible functional roles of cIgG. Initially, CA215 and cIgG were affinity-isolated from the shed media of a cultured ovarian cancer cell line, OC-3-VGH, respectively with RP215 and anti-human IgG as the general ligands.

The affinity-purified cognate antigens from the shed media of cancer cells were then used as general ligands to affinity-isolate any serum protein components that may be recognized by CA215 and/or cIgG. The affinity-isolated human serum proteins were further subjected to tryptic digestion and molecular analysis by LC-MS/MS [52]. Among the fifty detected serum protein components, more than 72% were found to be commonly recognized by both CA215 and cIgG. The majority of those human serum proteins have been previously identified as endogenous proteins which have been demonstrated to have either anti-cancer or pro-cancer properties [52]. They are listed in Table 2, according to their respective pro-cancer or anti-cancer properties. Details will be discussed in the following section.

CA215 (0.1 Au/ml)		Lung (n)	Liver (n)	Ovary (n)	Esophagus (n)	Breast (n)	Stomach (n)
CEA (5 ng/ml)	I	52% (112)	74% (58)	-	61%(23)	71% (44)	60% (30)
	II	64% (33)	54% (35)	-	47% (19)	95% (20)	50% (14)
	III	97%	81%	-	65%	96%	70%
AFP (20 ng/ml)	I	-	74% (58)	-	-	-	-
	II	-	50% (40)	-	-	-	-
	III	-	85%	-	-	-	-
CA125 (35 Au/ml)	I	52% (112)	74% (58)	59% (68)	61% (23)	-	-
	II	85% (13)	85% (13)	59% (66)	50% (12)	-	-
	III	85%	92%	82%	75%	-	-
CA19-9 (37 AU/ml)	I	-	74% (58)	-	-	-	60% (30)
	II	-	55% (22)	-	-	-	75% (16)
	III	-	82%	-	-	-	81%
CA15-3 (30 AU/ml)	I	-	-	-	-	71% (44)	-
	II	-	-	-	-	83% (6)	-
	III	-	-	-	-	83%	-
β_2 microglobulin (2.6 ng/ml)	I	-	74% (58)	59% (68)	-	-	-
	II	-	56% (16)	90% (10)	-	-	-
	III	-	81%	100%	-	-	-
Cyfra 21-1 (3.3 ng/ml)	I	52% (112)	-	-	-	-	-
	II	50% (52)	-	-	-	-	-
	III	77%	-	-	-	-	-

Table 1: Comparative positive detection rates of various cancers by CA215-based and other cancer-associated antigen-based enzyme immunoassay kits. I: CA215 only; II: other marker only; III: combined.

These experimental observations are consistent with our early hypothesis of two independent immune systems in the human body and dual roles of immunoglobulins expressed by cancer cells in general [53,54]. One is the traditional immune system, in which B and T cells play key roles in generating antibodies and TCRs with unlimited antigen-related diversity [18]. In contrast, the cancerous immunoglobulins can only be generated with limited hypermutations and no class switching in different cancer cells [43,44]. The limited number of expressed cIgG and/or CA215 may be sufficient to react with any key serum components for the growth/proliferation of cancer cells *in vivo*. At the same time, these cIgG may also serve to neutralize any anti-cancer serum proteins which may be harmful and lead to suppression of cancer cells in our normal human body microenvironment [52-54].

Therefore, it can be hypothesized that two separate and independent immune systems can exist in our human body. The normal one is used to fight off potentially harmful foreign pathogens. On the other hand, cIgG may serve dual functional roles for the growth/proliferation and immune-protection of cancer cells *in vivo* [52-54]. Therefore, any strategy resulting in the deactivation of this cancer immune system, including RP215 to target cIgG, should lead to effective immunotherapy of relevant cancer in humans.

Functional property	Protein (molecular weight)	Key notes
Pro-cancer	Complement factor H (139 kDa)	Expressed & secreted by many primary tumors & cancer cell lines to protect them from complement-mediated cytolysis [57-59,61,62] Downregulation sensitizes cells to complement attack & reduces tumor growth [57-60]
	Serotransferrin (77 kDa)	Important growth factor for cancer cell proliferation [76-78] Blocking serotransferrin binding to the cancer cell surface inhibits tumor growth in vitro [78,79]
	Vitronectin (54 kDa)	Inducer of cancer stem cell differentiation in breast & prostatic carcinoma [65,66] Interacts with the urokinase receptor to induce cancer cell spreading, migration, and growth [67,80]
	Others:	
	α2 macroglobulin (163 kDa)	[81,82]
	C4b binding protein (67 kDa)	[61,83]
	CD5 antigen-like protein (38 kDa)	[84]
	Complement C3 (187 kDa)	[63,64,85]
	Isoform 2 of α-1-antitrypsin (40 kDa)	[86]
	Hemopexin (52 kDa)	[87]
Anti-cancer	Anastellin	Inhibit tumor growth and metastasis *in vitro* through inhibiting angiogenesis [72,73] Inhibits serum dependent cell growth by blocking progression of the cell cycle [88]
	Apolipoprotein A-1	Biomarker of ovarian cancer [89] Net functional effects include a decrease in tumor growth, angiogenesis, metastasis, invasion, & myeloid-derived suppressor cell recruitment, as well as an increase in anti-tumor macrophages & CD8^{+} T cells cells [74] Apolipoprotein A-1-deficient mice develop tumors quicker than wild-type mice [74]
	Fibrinogen β chain	First 20 amino acids of the N terminus of the fibrinogen beta chain (β43-63) significantly inhibits VEGF-activated adhesion of epithelial cells to the extracellular matrix [71] In mouse models, inhibits tumor vascularization & increases tumor necrosis [71]
	Others:	
	35 kDa inter-α-trypsin inhibitor heavy chain 4 (104 kDa)	[69,90]
	Keratin type I cytoskeletal 9 (62 kDa)	[70]
	Complement component C4b (193 kDa)	[64,83]

Table 2: Human serum proteins or fragments detected commonly by CA215 and cIgG and cancer-related functional properties.

Human Serum Protein Components Recognized by CIgG and/or CA215

As stated in the previous section, serum proteins or components which show affinity to cIgG and/or CA215 were affinity isolated and analyzed by LC-MS/MS. They were then classified according to their known functional properties as "pro-cancer" or anti-cancer" [52]. Results of such analysis are presented in Table 2 for further investigations and discussions.

Among the pro-cancer serum components detected by CA215 or cIgG, the most notable ones include serotransferrin, C4b-binding protein, complement factor H, complement C3, and vitronectin [52].

Some of these pro-cancer serum components have been implicated as important growth factors for cancer cell proliferation, such as serotransferrin, the iron-binding transport protein within the human circulation [55,56]. Other pro-cancer serum components are members of the complement system, such as C4b-binding protein, complement factor H, and complement C3. Cancer cells are capable of binding to C4b-binding protein and complement factor H to protect cancer cells from complement activation [57-61]. In addition, many primary tumors and cancer cell lines have been known to express and secrete complement factor H [57-59,61,62]. Complement C3 has also been found to be synthesized by gastric-derived cancer cell lines [63]. Through production of vascular endothelial growth factor (VEGF) and extracellular matrix reorganization and disintegration, C3 has been

observed to promote cancer development and progression [64]. Vitronectin has been shown to be an inducer of cancer stem cell differentiation in breast and prostatic carcinoma, as well as an inducer of cancer cell spreading, migration, and growth [65-68].

Among the anti-cancer serum components detected by CA215 or cIgG, the most notable ones include 35 kDa inter-α-trypsin inhibitor heavy chain 4, anastellin, apolipoprotein A1, fibrinogen β chain and keratin type I cytoskeletal 9 [52]. Many of these anti-cancer serum components have been found to be downregulated in cancerous tissue, including 35 kDa inter-α-trypsin inhibitor heavy chain 4 and keratin type I cytoskeletal 9. Downregulation of 35 kDa inter-α-trypsin inhibitor heavy chain 4 in multiple solid tumors is believed to be associated with tumor initiation and progression [69]. Downregulation of keratin type I cytoskeletal 9 may be correlated with drug sensitivity of cancerous tissues [70]. Other anti-cancer serum components are also involved in inhibiting angiogenesis, such as the fibrinogen β chain, anastellin, and the cardioprotective protein, apolipoprotein A-1. In mouse models, the first 20 amino acids of the N terminus of the fibrinogen β chain (β43-63) haven been found to significantly inhibit tumor vascularization and increase tumor necrosis [71]. Anastellin, a fragment of the first type II module of fibronectin, has been found to inhibit tumor growth and metastasis *in vivo* through its inhibitory effects on angiogenesis [72,73]. Likewise, the cardioprotective protein, apolipoprotein A-1, which is a major protein component of high density lipoprotein (HDL), has been found to decrease angiogenesis, as well as decrease tumor growth, metastasis, invasion, and myeloid derived suppressor cell recruitment [74]. It has also been shown that mice lacking apolipoprotein A-1 were found to develop tumors quicker than wild-type mice [74].

Based on results of the results of this molecular analysis, it can be hypothesized that immunoglobulins expressed on the cancer cell surface may serve as receptors to interact with a variety of human serum protein components which exhibit pro- or anti-cancer properties. Therefore, through its direct or indirect interactions with human serum components, cIgG may serve dual roles in cancer cells by promoting cancer cell growth/proliferation, as well as immune protection of cancer cells from harmful agents within the human circulation.

Research and Development of RP215-Based Anti-Cancer Drugs

Since its discovery in 1987, RP215 has been utilized as a unique probe to study cIgG and/or CA215, including their biological/immunological characterization among cancer cells [20,41,42]. Subsequently, it was realized that the surface-bound cIgG and/or CA215 may be a potential target for cancer immunotherapy by RP215 Mab. RP215 was shown to induce apoptosis and CDC reactions when incubated with cancer cells *in vitro* and reduce tumor volume in nude mouse animal models [2,11,13,24,32]. The mechanisms of action of RP215's anti-cancer activity are more or less elucidated. Recently, humanized forms of RP215 have been successfully developed and shown to be bioequivalent to murine RP215 in terms of their relative biological actions in epitope specificity and affinity to cancer cells. Preclinical studies of humanized RP215 are being performed to see if it is qualified as antibody-based anti-cancer drug candidate for treatment of certain types of cancers in humans, especially for ovarian and lung cancer [75].

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