REVIEW ARTICLE

Asclepius

Biological Implications of Human Serum Proteins recognized by Cancerous Immunoglobulins

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ABSTRACT

Functional significance of cancerous immunoglobulins was evaluated and demonstrated through their binding interactions with human serum proteins. RP215 is a monoclonal antibody generated against ovarian cancer cell extract and reacts mainly with O-linked glycan-associated epitope in the variable regions of immunoglobulin heavy chains expressed by most of cancer cells in humans. They were designated in general, as CA215 but not structurally identical to those expressed normal B cells. RP215 was used as a unique probe to study interactions between human serum proteins and cancerous immunoglobulins. CA215 and cancerous immunoglobulins were affinity-isolated from the cancer cell extract, separately. Human serum proteins which react with CA215 and cancerous immunoglobulin (IgG expressed by cancer cells [CIgG]) were isolated by respective affinity columns. The purified human serum proteins were then subjected to liquid chromatography-mass spectrometry/mass (LC-MS/MS) analysis. Among the serum proteins or fragments, more than 80–85% were found to be identical between those isolated by CA215 and CIgG affinity columns. Further studies revealed that quite a few of these serum proteins were known to have pro-cancer or anticancer properties as studied by previous investigators. Based on these studies, it can be suggested that surface expressions of immunoglobulins by cancer cells are known to interact with certain human serum proteins with known anticancer or pro-cancer properties for growth/proliferation as well as survival/protein of cancer cells under our normal human environment.

Key words: A monoclonal antibody generated against ovarian cancer cell extract monoclonal antibody, anticancer serum proteins, cancerous immunoglobulins, pro-cancer serum proteins

INTRODUCTION

Immunoglobulins expressions by cancer cells were initially detected almost two decades ago, through gene expression studies of cancer cells with reverse transcriptase-polymerase chain reactions in 1998.[1,2] The functional roles and significance of those cancerous immunoglobulins remain to be elucidated. It was generally concluded from early studies that cancerous immunoglobulins are essential for growth/proliferation of cancer cells.[3] A major breakthrough came in 1987, when a monoclonal antibody RP215 was generated against ovarian cancer cell extract, OC-3-VGH ovarian cancer cell line.[4] Following matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis, the cognate antigen designated as CA215 was shown to consist mainly of cancer cell-expressed heavy chain immunoglobulins, with a carbohydrate-associated epitope recognized by RP215 in the variable regions.[4] Further biological and immunological studies revealed that RP215 and anti-immunoglobulins act similarly to induce apoptosis and complement-dependent cytotoxicity to cancer cells *in vitro* and inhibit tumor growth *in vivo* through mude mouse experiment.[4-6] Furthermore, comparative gene regulation studies revealed that RP215 and anti-human IgG affect the levels of gene regulations similarly to cancer cells *in vitro* with high degree of correlations. Therefore, RP215 can be used as a suitable probe to study the interactions between human serum protein and cancerous immunoglobulins. From the results of such studies, it is possible to evaluate functional

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significance of immunoglobulins universally expressed by cancer cells.[7]

ISOLATION OF CA215 AND CANCEROUS IMMUNOGLOBULINS AND REACTIVE HUMAN SERUM PROTEINS

Interactions between human serum proteins and cancerous immunoglobulins can be best achieved by affinity chromatography followed by liquid chromatography-mass spectrometry/mass (LC-MS/MS) analysis to reveal the molecules nature of these proteins and its biological implications to cancer cells.[7] Initially, CA215 and cancerous immunoglobulins (CIgG) were affinity isolated using RP215 and anti-human IgG which were used as separate ligands. Subsequently, purified CA215 and CIgG were used as affinity ligands to isolate those interactive human serum proteins. The sodium dodecyl sulfate-page analysis of CA215 and CIgG is shown in Figure 1 for comparisons.

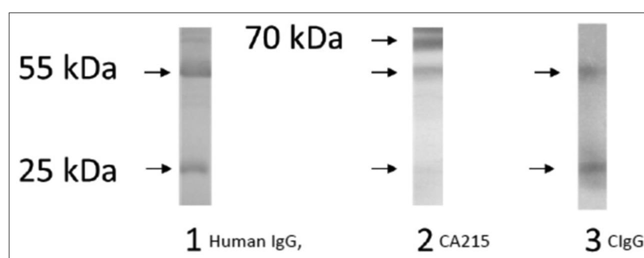

Figure 1: SDS - PAGE (10% gel) to reveal the molecular size of protein bands of affinity purified CA215 recognized by a monoclonal antibody, RP215 generated against ovarian cancer cell extract (10 μg/gel) and IgG expressed by cancer cells (10 μg/gel). The molecular weight marker of protein bands is indicated by arrows 25 kDa, 55 kDa and 70 kDa, respectively

Enzyme immunoassay was performed to reveal specific binding between CA215 or CIgG and purified human serum proteins (designated as CA215-s and CIgG-s, respectively). Following affinity purification, CA215-s and CIgG-s were coated on microwells, and biotinylated CA215 and CIgG were added dose dependently for subsequent incubations. The degree of dose-dependent interactions between CA215-s or CIgG-s and CA215 or CIgG was demonstrated by adding alkaline phosphatase-labeled avidin as the capturing antibodies. The results were presented in Figure 2 for comparisons.[7]

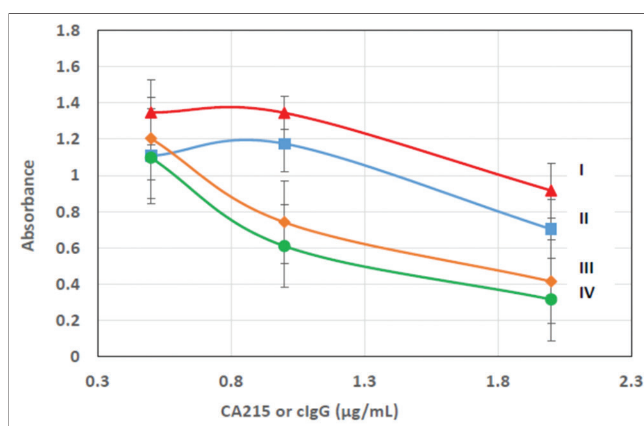

Figure 2: Enzyme immunobinding assay to reveal the binding of CA215 recognized by RP215 monoclonal antibody generated against ovarian cancer cell extract (CA215) and IgG expressed by cancer cells (CIgG-s) to CA215 or CIgG, respectively. CA215-s (lots I and II) and CIgG-s (lots III and IV) were coated, respectively, on microwells at a concentration of 1 μg/mL. Affinity-purified CA215 and CIgG were biotinylated as CA215-b and CIgG-b, respectively. CA215-s and CIgG-s-coated wells were incubated with different concentrations of CA215-b and CIgG-b, respectively, for 3-h incubations at 37°C. Following wash alkaline-phosphatase-labeled avidin was added to each well for additional 60 min incubation. The color development was made at the end of incubation and wash, and the absorbance at 405 nm was determined in duplicates at different concentrations of CA215-b and CIgG-b. The data are presented in duplicates following subtraction of negative control. Dose-dependent bindings were statistically significant.

ANALYSIS OF SERUM PROTEINS RECOGNIZED BY CA215 AND CIgG

The affinity-isolated CA215s or CIgGs were then concentrated and freeze-dried for LC-MS/MS analysis.[7] Hundreds of human serum proteins interacting with CA215 and/or CIgG are identified separately. The commonly detected human serum proteins account for as many as 85% of the total proteins identified by either CA215 or CIgG as the affinity ligand. Almost all of the identified proteins have been studied previously related to their pro-cancer or anti-cancer activities. Some of these proteins were highlighted and discussed as follows, according to biological natures of their properties.

Pro-cancer serum proteins

Among the isolated/detected pro-cancer serum protein components, five were found to be the notable ones. These include (I) 4b-binding protein α chain, (II) complement C3, (III) complement factor H, serotransferrin, and (V) vitronectin.

C4b-binding protein (67 kDa) is known to be a soluble complement inhibitor by blocking complement classical pathway. Cancer cell protections have been demonstrated in the case of SKOV-3 and CAOV-3 ovarian cancer cell lines by this serum protein.[8,9]

Complement C3 (187 kDa) is an essential protein involved in the complement cascade.[10-12] Its clavage products were shown to demonstrate proliferation activities associated with

disintegration for tumor angiogenesis. Cancer development and progression can be promoted by this complement protein.[10-12]

Complement factor H (139 kDa) is a key regulatory serum glycoprotein in the alternative complement system. It is frequently expressed by many tumors and cancer cell lines, such as glioblastomas and several other carcinomas to resist complement-mediated cytolysis.[13-15] Therefore, complement factor H can act to protect cancer cells from complement activation.[15]

Serotransferrin (77 kDa) is an iron-binding protein in human serum and plays an essential role in various cellular functions such as energy metabolism and cell growth or proliferation.[16] Blocking transferrin receptor on cancer cell surface can result in the inhibition of cancer cell proliferation.[17,18]

Vitronectin (54 kDa) is a liver glycoprotein and was shown to induce stem cell differentiations in breast and prostate cancer.[19] Therefore, migration and growth of cancer cells in human serum environment can be promoted.[20]

Anticancer serum proteins

Among the human serum proteins which are recognized by CIgG or CA215, quite a few were known to be anticancer in nature. Among the list are (I) inter-α-trypsin inhibitor, (II) anastellin, (III) apolipoprotein A1, (IV) fibrinogen β chain, and (V) keratin type 1 cytoskeletal 9.[21]

Inter-α-trypsin inhibitor heavy chain 4 (104 kDa) produced by the liver was known to exhibit anticancer properties.[22] The downregulation of this serum protein by small RNA of interference (SiRNA) can lead to the initiation and progression of tumors.[23]

Anastellin (256 kDa) is a fragment of the first Type III module of fibronectin.[24] It is capable of inhibiting tumor growth and metastasis through its inhibitory effects on angiogenesis and blockage of cell cycle progression.[25]

Apolipoprotein A-1 (31 kDa) is a major protein component of high-density lipoprotein in human plasma with anti-inflammatory and antioxidant properties.[26] It was shown to suppress tumor growth, metastasis, and angiogenesis in animal model studies. Furthermore, suppression of apolipoprotein A-1 expression could facilitate tumor development in mice.[26]

Fibrinogen β chain (56 kDa) is one of three peptide chains that form fibrinogen and was known to be involved in blood clot.[27] It was found to inhibit tumor vascularization and to increase tumor necrosis.[28] Keratin type I cytoskeletal 9 (62 kDa) is known to be associated with drug resistance in breast cancer when this protein is downregulated.[21]

BIOLOGICAL IMPLICATIONS OF INTERACTIONS BETWEEN CANCEROUS IMMUNOGLOBULIN AND HUMAN SERUM PROTEINS

From this short review, it has become apparent that significant differences exist between the conventional immunology and cancer immunology. In the case of conventional immunology, both B and T cells were derived, separately to coordinate and maintain acquired immunity against foreign pathogens. In contrast, among cancer cells, expressions of immunoglobulins seem to be essential for the growth/proliferation as well as for survival of cancer cells under normal human environment.[3,4]

Early studies of cancerous immunoglobulins revealed that apoptosis can be induced by the presence of anti-immunoglobulins or RP215 as well as immunoglobulin-related SiRNA.[3] These experimental observations provide evidence to the essential requirements of cancer cell-expressed immunoglobulins among almost all cancer cells.[4] Furthermore, dual functional roles of cancerous immunoglobulins were clearly demonstrated from studies of interactions between human serum proteins and cancerous immunoglobulins as described in this review. Following comprehensive LC-MS/MS analysis of affinity-isolated serum proteins, quite a few were shown to exhibit either pro-cancer or anticancer properties.[7] Therefore, it is logical to assume that these specific human serum proteins can interact with cancer cells for their growth/survival under our normal human serum environments.

These specific cancerous immunoglobulins might be expressed through somatic mutations, of immunoglobulin genes of cancer cells, similar to those of B cells in the conventional immunology. However, cancerous immunoglobulin is expressed entirely different from those of conventional immunology, of which B cells and T cells as well as foreign pathogens are involved. Nevertheless, the molecular mechanisms for expressions of cancerous immunoglobulins remain to be investigated.

From this study, we were given the fact that pro-cancer and anticancer of human serum proteins or fragments are involved in proliferation/growth and survival/protections, of the cancer cells under our normal human environment.[7] Therefore, it is important to further investigate the physiological levels of these serum proteins and their relationships with cancer in humans. We believe that immunodiagnostics of these serum proteins should be beneficial to cancer diagnostics and preventions.[7]

Although, in this report, only a few human serum proteins were highlighted, we believe that more of specific serum proteins are involved in the complicated interactions with human cancer cells under our normal human body environments. The expressions of cancerous immunoglobulins on cancer

cell surface may serve the essential purposes for growth/survival of cancer cells. Its biological implications should deserve further in-depth investigations.

Abbreviations

CA215: Tumor-associated antigen 215 recognized by RP215
cIgG: IgG expressed by cancer cells
LC-MS/MS: Liquid chromatography-mass spectrometry/mass
MALDI-TOF MS: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry
OC-3-VGH: Ovarian cancer cell line of serous origins from Dept CBS/GYN, VGH, Taipei Taiwan
RT-PCR: Reverse transcriptase polymerase chain reactions
RP215: A monoclonal antibody generated against ovarian cancer cell extract
SiRNA: Small RNA of interference
SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel

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