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Distinct functional roles of cancerous immunoglobulins in cancer immunology

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Abstract

The immune system in cancer cells was revealed with the understanding that immunoglobulins expressed on the cancer surface play important roles in cancer immunology. RP215 monoclonal antibody generated in 1987 against ovarian cancer cell extract was shown to react specifically with a carbohydrate-associated epitope mainly found in the variable region of immunoglobulin heavy chains and expressed on the surface of almost all of cancer cells (designated in general as CA215). Since then, RP215 has become a unique probe to study mechanisms of action by which the cancer cells are affected by these immunoglobulins. Generally speaking, RP215 and anti-human immunoglobulins are equally effective in inducing apoptosis and complement-dependent cytotoxicity reactions to cultured cancer cells and reducing the volume of the implanted tumor in nude mouse animal models. Interaction studies were performed between isolate cancerous immunoglobulins and/or CA215 and human serum proteins, most of which exhibit either anti-cancer or pro-cancer properties. Therefore, it is hypothesized that cancerous immunoglobulins may function to interact with these human proteins for the growth/proliferation as well as protections of cultured cancer cells in human circulations. RP215 may be further developed as candidates of anti-cancer drugs to target most of cancer cells for immunotherapy of human cancer.

Introduction

It is known for decades that immunoglobulins can be expressed by cancer cells of epithelial origins [1]. Early studies seemed to suggest that surface-expressed immunoglobulins are essential for growth and proliferation of cancer cells *in vivo*. This conclusion was drawn based on the immunoglobulins-related SiRNA studies [2,3], whereas the interference of immunoglobulin gene expression by SiRNA can result in depression of cancer growth and/or proliferation [1,4,5]. A major breakthrough came in 1987, when a monoclonal antibody designated as RP215 was generated against the cell extract of an ovarian cancer cell line of serus origin (OC-3-VGH) [4,5]. Through comprehensive analysis by MALDI-TOF MS (matrix-assisted laser desorption ionization time-of-flight mass spectrometry), the purified cognate antigen (designated in general as CA215) was found to consist mainly of immunoglobulins heavy chains expressed by cancer cells, but not found in those of B-cell origins [6,7]. This monoclonal antibody has become a unique probe to study functional roles of cancerous immunoglobulins for numerous biological and immunological studies. The main objective of those studies is to determine its mechanisms of action against human cancer *in vitro* and *in vivo* and the suitability of RP215 to serve as candidate for anti-cancer drug developments. Therefore, in this short review, distinct roles of cancerous immunoglobulins will be highlighted through the use of RP215 as the tool for such investigations [8-10].

Epitope recognized by RP215 is carbohydrate-associated

There are numerous indirect experimental evidences to indicate that RP215-specific epitope is carbohydrate-associated [11,12]. Initial experimental observations showed that mild periodate treatments result in loss of RP215 binding activity in CA215 [1], so is the heat treatment (100 ^{0}C for 5 min), indicating carbohydrate's involvement in the epitope recognition. Upon continuous culture of cancer cells in serum free medium in the absence of carbohydrate precursors (except glucose), a significant loss of CA215 immunoactivity was observed [10,13]. Furthermore, culturing of cancer cells in human serum instead of bovine serum albumin does not result in loss of CA215 immunoactivity, suggesting that NeuAc or NeuGc does not alter the binding of RP215 to CA215. Through DNA analysis of IgG genes from cancer cells, the amino acid sequence of the Fc region of cancerous immunoglobulins is almost identical to that of normal human IgG, suggesting that the epitope recognized specifically by RP215 may be carbohydrate-associated and not due to gene variations [14,15]. This RP215-specific epitope can also be detected among many other glycoproteins having no apparent sequence homology to human immunoglobulins such as those of immunoglobulin superfamily proteins (IgSF) having similar immunoglobulin fold. Based on the results of binding ELISA's, the preferential location of RP215-specific epitope in CA215 or cancerous immunoglobulins should be on the Fab variable domains of the IgG heavy chains. In view of the high binding affinity between RP215 and CA215, it is therefore suggested that amino acid residues of the protein backbones may be involved to constitute RP215-specific epitopes [16,17]. The RP215 binding epitope in CA215 may consist of only one typical glyco-conjugate. Based on our experimental observations, the apparent binding affinity of RP215 to various cancer cell lines including OC-3-VGH (ovarian), C33A (cervical) and ME-180 (cervical) coated on microwells was found to be almost identical when ELISA binding assays were performed (Kd 2-5 nM). Among CA215 recognized by RP215, the majority of glycopeptides isolated were found to be those of cancerous immunoglobulins based on results from comprehensive analysis of glycopeptide mapping (80-

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90%) [10]. RP215-specific carbohydrate-associated epitope may be O-linked glycan located in the variable regions of immunoglobulins. This conclusion was drawn based on the fact that tunicamycin, inhibitor of N-glycan biosynthesis has little effect of RP215-specific immunoactivity following continuous culture of cancer cells in the presence of the inhibitor [6,18,19]. Based on the results of glycosyl-linkage analysis, only two simple O-linked glycans were identified and tentatively assigned for cancerous immunoglobulins and/or CA215. The O-linked glycans from CA215 and cancerous immunoglobulins contain tri-saccharides with terminal N-acetyl neuraminic acid (NeuAc) and was found to be a major glycan component. The glycosyl linkage analysis indicates that the main O-glycans are Core-1 structure with 3-linked and 3, 6-linked GalNAcitol [GalNAc-Gal (3)-NeuAc (6)] [13,15,20].

In contract to normal mouse IgG or human IgG, the affinity-isolated cancerous IgGs were found to have relatively low immunoactivity, suggesting that the cancerous IgG may be aberrantly glycosylated in the IgG variable region [21,22]. By summarizing all the experimental observations, it can be concluded that RP215-specific epitope are most likely associated with O-linked glycans predominately on the variable domains of the heavy chains of cancerous immunoglobulins and many others such as IgSF-related or unrelated glycoproteins [1,23]. The O-linked glycans are of Core 1 structure and can be redefined as T-S antigen. This newly found T-S antigen-epitope is apparently highly tumor-associated and is distinct from T, Tn or STn antigen (tumcated O-linked glycans) which were commonly found to link on cancer-associated mucins and gangliosides [24-28]. From the above experiments, it can be concluded that RP215 reacts mainly with the epitope located on the variable domains of cancerous immunoglobulins. The O-linked glycans of Core 1 structures are clearly involved in the epitope recognition by RP215. Normal immunoglobulins and cancerous immunoglobulins in human body are clearly differentiated by the aberrant O-linked glycans of the later which is recognized by RP215 monoclonal antibody [7,12,29,30].

Functional studies of cancerous immunoglobulins

In vitro and *in vivo* studies

Since the discovery of RP215 in 1987, little was known about the molecular nature of RP215-specific epitope and the cognate antigen, CA215 [6]. However, the partial immunodiagnostic applications of CA215 as pan cancer biomarker were realized and reported in preliminary clinical studies for ovarian and cervical cancer [31,32]. Almost twenty years later, CA215 was isolated from shed medium of cultured cancer cells with RP215-related immunoaffinity chromatography [7,32]. The molecular identity of CA215 analyzed by MALDI-TOF MS method [8,9] was eventually revealed. It was found that CA215 consists mainly of immunoglobulins expressed by most cancer cells of epithelial origins, but not found in those of normal B cell origin [33,34]. Further analysis also indicated that CA215 was defected not only in cancerous immunoglobulins but also many other glycoproteins such as immunoglobulin superfamily proteins (IgSF), including T cell receptors, MHCI and MCHII as well as cell adhesion molecules, which account for more than 60% detected [35]. Immunohistochemical studies revealed that RP215 can react with the surface of many cancer cells either in cell lines or cancerous tissue sections [10,14,23,36]. Antibodies against human immunoglobulins can also react with many cancer cells on the surface. These results suggest that both anti-human IgG and RP215 can interact specifically with the surface-expressed cancerous immunoglobulins of cancer cells from different tissue origins [37-39].

Effects of RP215 and anti-human IgG were studied with cultured cancer cells, including ovarian (OC-3-VGH) and cervical (C33A) cancer cell lines and many others [1,40,41]. With almost no exceptions, apoptosis was induced to cultured cancer cells when incubated with either murine RP215, humanized RP215 or anti-human IgG at a concentration of 1-10 μg/ml in culture for 24-48 hours [17, 42-44]. Upon incubation of RP215 with cultured cancer cells in the presence of complement, complement-dependent cytotoxicity reactions (CDC) can be induced to all cultured cancer cells.

"Proof of concept" experiments were demonstrated by nude mouse animal models. Significant reductions of tumor volume were observed when RP215 was injected to nude mice with either OC-3-VGH ovarian or SK-MES-1 lung cancer cell lines in a dose-dependent manner [34, 45-47].

Effects of RP215 on gene regulations of cancer cells

Mechanisms of action by which the tumor growth was inhibited *in vitro* and *in vivo*, by RP215 were elucidated through gene regulation studies [27,48-50]. By means of semi-quantitative PCR, as many as one dozen of genes involved in growth/proliferation as well as innate immunity of cancer cell were employed for such investigations. Among these are P1 (for protein synthesis), P21, cyclin D1, c-fos (cell cycle regulations), and IgG, NFkB-1 (IgG expression) as well as toll-like receptors such as TLR-3, TLR-4 and TLR-9. The effects of treatments of RP215 and anti-human IgG, respectively on regulation changes of the selected genes were determined and correlated. It was observed the correlation coefficient ($R2 = 0.957$) between these two anti-antigen receptor ligands is excellent. This would suggest that RP215 can be a suitable substitute for antibodies against human IgG and can be used to target cancer cell surface-expressed immunoglobulins. Therefore, RP215 can be developed as an excellent candidate of anti-cancer drug development for therapeutic applications in various human cancer [1,17,51].

Interactions between CA215 or cancerous IgG and human serum protein components and immunological implications

Normal *vs.* cancerous immunoglobulins

The aberrant O-linked glycans identified on cancerous immunoglobulins were specifically recognized by RP215. Distinct differences in functions and expressions were observed between cancerous immunoglobulins and normal immunoglobulins derived from B cells [21,22,52]. In case of normal antigen receptors, such as immunoglobulins and T cell receptors, they are expressed by B cells and T cells, respectively in our body immune system. In contrast, both immunoglobulins and T cell receptors are co-expressed by the same cancer cells, even those derived from the same clonal origin. In case of normal immunoglobulins, they were derived from B cells through class switching and high frequency of hyper mutation, whereas for expression of cancerous immunoglobulins, such expression mechanisms either do not exist or very limited [53]. No O-linked glycans or aberrant glycosylations were found in normal immunoglobulins, except the common N-glycosylation at N297 position of IgG heavy chains with terminal NeuAc. In normal human tissues, only NeuAC is found, but in cancerous tissues, both NeuAC and NeuGC are found [1]. Recognition sites by RP215 Mab was never found in normal IgG [21,54]. In contrast, both O-linked and N-linked glycans sere detected

in the cancerous IgG heavy chain with terminal NeuAC and/or NeuGc. Such an aberrant O-linked glycosylation with Core 1 tri-saccharide structure of "T-S" antigen epitope may result in generation of unique protein-carbohydrate combined epitope recognized by RP215. Due to the aberrant O-linked glycosylations of cancerous immunoglobulins, low immunoactivity binding activity was observed as described previously when compared to that of normal immunoglobulins (with anti-human IgG as the binding probe). Furthermore, bindings of cancerous immunoglobulins on the surface of cancer cells can result in dramatic regulation changes of many genes involved in growth/proliferation as well as innate immunity such as toll-like receptors of cancer cells. In contrast to normal B cells which produce and secrete immunoglobulins, such gene interactions or regulations have never been reported or found [50,55,56]. Finally, apoptosis and complement-dependent cytotoxicity (CDC) can be induced upon treatments of cultured cancer cells with anit-antigen receptors or RP215, whereas no such mechanisms exist in normal B cells.

Based on these observations, it can be hypothesized that both normal immune and cancer immune systems are distinct and can co-exist in our body environment.

Dual distinct rolls of cancerous immunoglobulins through interactions with human serum proteins

The interactions between cancerous immunoglobulins and human serum proteins were investigated to elucidate their functional roles in cancer immunology. Initially, CA215 and cancerous immunoglobulins were isolated by affinity chromatography with RP215 and anti-human IgG as the affinity ligands, respectively from shed medium of cultured cancer cells (OV-3-GH, ovarian) [41,57]. The affinity-isolated CA215 and cancerous immunoglobulins were then employed as ligands to isolate human serum proteins which show any binding affinity to CA215 and/or cancerous immunoglobulins, respectively [48,58]. The isolated proteins were subject to comparative analysis by LC-MS/MS methods. The results of such comparative analysis suggest that as many as 80-86% of the isolated human serum proteins were identical between those purified by CA215 and by cIgG affinity columns, respectively. Among dozens of proteins isolated, they can be classified, in general, by their intrinsic pro-cancer or anti-cancer properties. Among the pro-cancer human serum proteins commonly recognized by CA215 and/or cIgG are C4 binding proteins, α-chain, complement C3 and complement factor H, serotransferrin and vitro nectin [59-62]. On the other hand, the following were identified as anti-cancer human serum proteins including apolipoprotein A1, fibrinogen β chain, inter-α-trypsin inhibitor heavy chains 4, anastellin, keratin type 1 cytoskeletal 9 and anti-immune IgG [59-62].

These experimental observations have strongly suggested two distinctly different functional roles of cancerous immunoglobulins are simultaneously played by cancer cells in cancer immunology [4,8,47]. Therefore, we believe that cancerous immunoglobulins can serve as specific binding proteins to capture some human serum proteins to promote growth/proliferation of cancer cells [35,63,64]. At the same time, these cancerous immunoglobulins are also able to adsorb, neutralize or interact those with anti-cancer properties from human circulations for the survival of cancer cell in our normal human body environment [25, 61-63,65].

The exact mode of interactions between selected human serum components and cancerous immunoglobulins on cancer cell surface and remains to be explored by further investigations [57,58,66]. The possibility of signal transductions leading to dramatic changes in gene regulation of cancer cells cannot be ruled out without further in-depth studies.

Potential immunodiagnostic and immunotherapeutic applications of cancerous immunoglobulins

CA215 and/or cancerous immunoglobulins in immunodiagnostic applications

Since RP215 was shown to be more or less bioequivalent to anti-human IgG in biological and immunological behaviors, RP215-based immunodiagnostic applications can be expected to determine cancer immunoglobulins or CA215 in cancer patient's serum samples [56,67,68].

During early clinical studies, serum CA215 levels for patients with cervical or ovarian cancer were monitored by RP215-bassed enzyme immunoassay kits. It was generally observed that elevated serum levels of CA215 are cancer stage-dependent in either cervical or ovarian cancer patients [9,69-71]. It was clearly demonstrated that positive detection rates of CA215 are well correlated with cancer stages [72,73]. For example, in the case of cervical cancer, the positive detection rates can be as high as 66-94% at different cancer stages [56,74]. Similarly, for ovarian cancer, the positive rates range from 58-86% and also are stage-dependent. However, state-dependence of CA215 levels in either case, are more significant between stage I and stage II or stage III [1,58,66,75]. In a separate study, CA 215 levels of given cancer patients were monitored during the course of medical treatments. It was found that seven days after surgical treatments or chemotherapy, CA215 levels were found to decrease significantly, when compared to those without any treatments [76]. Therefore, CA215 enzyme immunoassay kit can be beneficial for routine monitoring of cancer patients who show positive CA215 levels [32,77].

More than 500 serum specimens from patients confirmed with different cancers were employed for evaluations of serum CA215 levels by the established CA215 EIA kit [1,34,50]. The CA215 levels of these serum specimens were then compared with those of other cancer biomarkers for relative rates of positive detections [11]. Based on the results of RP215-based enzyme immunoassays, it was concluded that the positive detection rates of CA215 among different cancers are generally higher than 50% when compared with those of the normal individuals [68,78]. Among these are lymphoma (83%), or the cancer of lung (52%), liver (74%), esophagus (61%), stomach (60%), ovary (59%), breast (71%), and cervix (51%). Side by side comparisons were made with other known cancer biomarkers such as CA215, CA15-3, CA19-9, AFP, CEA and cyfra21-1 and β2 microglobulins [33,79]. It was clearly demonstrated, when CA215 was combined with other types of biomarkers for cancer detections, much higher positive rates were found [50,67]. For example, by combining both CA215 and CA125, positive detection rates can increase from 59% to 82% for patients with ovarian cancers as judged from results obtained from clinical studies in three separate major medical centres [26,34].

Cancerous immunoglobulins and immunotherapeutic applications

Immunoglobulins expressed on the surface of most cancers can be a unique target for RP215 which specifically recognizes cancerous immunoglobulin. Previous biological and immunological studies also indicated that RP215 could induce apoptosis to cultured cancer cells and CDC reactions in the presence of complement to induce lysis of cultured cancer cells [16,52,80]. Injections of RP215 to tumor-

implanted mice can also result in significant reductions of tumor volume in a dose-dependent manner as revealed by several typical nude mouse animal models [37,75].

Basically, two strategies are currently available for the development of RP215-based anti-cancer drugs. One is passive immunization of humanized forms of RP215 in gram or subgram quantity to cancer patients for treatment. The other is CAR-T cell therapy. Humanized RP215 genes in the Fab domains are packaged into a vector in a form of chimeric antigen receptors (CAR). Transfection of patients' own T cells with CAR-related vector for further expansion of T cells can result in RP215 expression to target cancer cells *in vivo*. RP215-related CAR-T system may represent a promising approach for cancer immunotherapy in the future [1,11,81].

RP215 which is of mouse origin can be engineered to humanized forms, especially the Fab regions in the FR domains of both IgG heavy and light chains. Alterations of amino acid sequence of CDR regions should be minimized due to the critical antigen binding domains [81,82]. The humanized antibodies of RP215 may be assembled in different forms including whole antibodies, fragments such as Fab, Fab' and $(Fab')_2$ as well as recombinantly produced single chain antibodies (scFv), the last of which are commonly used in CAR-T technology and immunoreactive to CA215. The resulting humanized RP215 should be of equivalent or comparable affinity and specificity to the original murine RP215 and usually contain substantially similar or identical CDRs regions or domains [81]. Stable CHO cell lines secreting humanized RP215 are being developed for large scale productions of humanized RP215 by *in vitro* culture [11,41]. Preclinical studies by using humanized RP215 are being carried out to meet the criteria of IND (initial new drug development) as required by US FDA [83,84].

Conclusions

In this short review, we have summarized results of our recent studies on cancerous immunoglobulins to elucidate their mechanisms of action in cancer immunology [32,41,42] and potential clinical applications. Immunoglobulins produced by cancer cells are different from those of B cell origins. Aberrant glycosylations in cancerous immunoglobulins result in creation of a unique carbohydrate-associated epitope recognized by RP215 monoclonal antibody. RP215 can therefore become a useful tool for studies of cancerous immunoglobulins. Through years of investigation, we begin to realize that two distinct functional roles may exist for cancerous immunoglobulins. In general, under our normal body environment, many of human serum proteins are commonly recognized by cancerous immunoglobulins or RP215 and are known to possess either anti-cancer or pro-cancer properties, *in vitro* or *in vivo* [21,22]. Therefore, it is reasonable to hypothesize that cancerous immunoglobulins are essential for the growth / proliferation of cancer cells under our normal body environment by means of interactions or binding with certain human serum proteins. These human serum proteins have been shown previously by others to promote the cancer cell to grow or proliferate upregulation of growth factors, and down regulation of complement attack to cancer cells as well as angiogenesis to cancer cells [57,79].

On the other hand, interactions with certain human serum proteins may result in suppression to the growth of cancer cell. Cancerous immunoglobulins may serve to neutralize those hostile proteins or components in human circulations. Therefore, RP215 which targets surface-expressed cancerous immunoglobulins may become a unique choice for cancer immunotherapy. Preclinical and clinical studies of humanized RP215 are required for further anti-cancer drug development. It is expected that humanized RP215 may become effective anti-cancer drugs either by passive immunization or by RP215-related CAR-T cell therapy in the near future [1,10].

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References

1. Lee G (2014) Cancerous Immunoglobulins in Cancer Immunology. *J Clin Cell Immunol* 05: p. 1-8.
2. Triplett A, Hurwitz AA (2014) Complement and Adaptive Immunity: Roles for the Anaphylatoxins C3a and C5a in Regulating Tumor Immunity. International Trends in Immunity 2(2).
3. Pio R, Corrales L, Lambris JD (2014) The role of complement in tumor growth. *Adv Exp Med Biol* 772: 229-262. [Crossref]
4. 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. [Crossref]
5. Pilzer D, Saar M, Koya K, Fishelson Z (2010) Mortalin Inhibitors Sensitize K562 Leukemia Cells to Complement-Dependent Cytotoxicity. *Int J Cancer* 126: 1428-35. [Crossref]
6. Lee G, Laflamme E, Chien CH, Ting HH (2008) Molecular identity of a pan cancer marker, CA215. *Cancer Biol Ther* 7: 2007-2014. [Crossref]
7. Lee G, Ge B, Huang T-K (2009) Positive Identification of Ca215 Pan Cancer Biomarker from Serum Specimens of Cancer Patients. *Cancer Biomark* 6: 111-7. [Crossref]
8. 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: 148-54. [Crossref]
9. 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. [Crossref]
10. 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.
11. 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. [Crossref]
12. 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: 1347-56.
13. Lee G, Chu RA, Ting HH (2009) Preclinical assessment of anti-cancer drugs by using RP215 monoclonal antibody. *Cancer Biol Ther* 8: 161-166. [Crossref]
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. [Crossref]
15. Lee G, Ge B (2009) Cancer Cell Expressions of Immunoglobulin Heavy Chains with Unique Carbohydrate-Associated Biomarker. *Cancer Biomark* 5: 177-88. [Crossref]
16. Lee G, Huang C, Zhang H, Tang Y (2014) The Relationships between Toll-Like Receptors and Rp215-Associated Immunoglobulins Expressed by Cancer Cells. *J Cancer Sci Therapy* 6: 77-80.
17. 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.
18. Lee G, Wu Q, Li C, Ting H, Chin-Hsiang C (2006) Recent Studies of a New Carbohydrate-Associated Pan Cancer Marker, Ca215. *J Clin Ligand Assay* 29: 47-51.
19. Lee G (2009) Cancer Cell-Expressed Immunoglobulins: Ca215 as a Pan Cancer Marker and Its Diagnostic Applications. Cancer Biomarkers 5:137-42. [Crossref]
20. Lee CY, Chen KW, Sheu FS, Tsang A, Chao KC, et al. (1992) Studies of a tumor-associated antigen, COX-, recognized by a monoclonal antibody. *Cancer Immunol Immunother* 35: 19-26. [Crossref]

21. Lee G, Huang C and Liu S (2014) Human Serum Proteins Recognized by Ca215 and Cancerous Immungobulins and Implications in Cancer Immunology. *J Cancer Clin Oncol* 3: 51-69.

22. Lee G, Huang C, Liu S, Chien C (2014) Dual Roles of Cancer Cell-Expressed Immunoglobulins in Cancer Immunology. *Amer J Immunol* 10: 156-165.

23. Lee G, Cheung AP, Li B, Ge B, Chow PM (2012) Molecular and immuno-characteristics of immunoglobulin-like glycoproteins in cancer cell-expressed biomarker, CA215. *Immunol Invest* 41: 429-446. [Crossref]

24. Smorenburg SM, Griffini P, Tiggelman AM, Moorman AF (1996) A2-Macroglobulin Is Mainly Produced by Cancer Cells and Not by Hepatocytes in Rats with Colon Carcinoma Metastases in Liver. *Hepatology* 23: 559-570. [Crossref]

25. Rutkowski MJ, Sughrue ME, Kane AJ, Mills SA, Parsa AT (2010) Cancer and the complement cascade. *Mol Cancer Res* 8: 1453-1465. [Crossref]

26. Díez-Itza I, Vizoso F, Merino AM, Sánchez LM, Tolivia J, et al. (1994) Expression and prognostic significance of apolipoprotein D in breast cancer. *Am J Pathol* 144: 310-320. [Crossref]

27. Johnson LD, Goubran HA, Kotb RR (2014) Histidine rich glycoprotein and cancer: a multi-faceted relationship. *Anticancer Res* 34: 593-603. [Crossref]

28. 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. [Crossref]

29. Lee CY, Lappi DA, Wermuth B, Everse J, Kaplan NO (1974) 8-(6-Aminohexyl)-amino-adenine nucleotide derivatives for affinity chromatography. *Arch Biochem Biophys* 163: 561-569. [Crossref]

30. Lee G, Cheung A, Ge B (2010) Monoclonal Anti-Idiotype Antibodies against Carbohydrate-Associate Epitope for Anti-Cancer Vaccine Development. *J Vaccines Vaccination* 1.

31. Lee G, Ge B (2010) Growth Inhibition of Tumor Cells in Vitro by Using Monoclonal Antibodies against Gonadotropin-Releasing Hormone Receptor. *Cancer Immunol Immunother* 59: 1011-9. [Crossref]

32. Lee G (2012) Cancerous Immunoglobulins and Ca215: Implications in Cancer Immunology. *Amer J Immunol* 8: 101-16.

33. Huang L, Gao J, Pu C, Zhang PH, Wang LZ, et al. (2015) Apolipoprotein M: Research Progress, Regulation and Metabolic Functions (Review). *Mol Med Rep* 12: 1617-24. [Crossref]

34. 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. [Crossref]

35. Rutkowski S, von Hoff K, Emser A, Zwiener I, Pietsch T, et al. (2010) Survival and prognostic factors of early childhood medulloblastoma: an international meta-analysis. *J Clin Oncol* 28: 4961-4968. [Crossref]

36. Lee G, Ge B (2012) Implications of Apoptosis in Cancer Immunotherapy. *Advances Biosci Biotech* 3: 679-85.

37. Lee G, Zhu M, Ge B (2012) Potential Monoclonal Antibody Therapy for the Treatment of Ovarian Cancer. Ovarian Cancer - Basic Science Perspective. Farghaly SA: InTech. 385-406.

38. Lee G, Zhu M, Ge B, Cheung AP, Chien CH, et al. (2012) Carbohydrate-associated immunodominant epitope(s) of CA215. *Immunol Invest* 41: 317-336. [Crossref]

39. Lee G, Zhu M, Ge B, Potzold S (2012) Widespread Expressions of Immunoglobulin Superfamily Proteins in Cancer Cells. *Cancer Immunol Immunother* 61: 89-99. [Crossref]

40. Lee G, Huang C, Chow S, Chien C, (2013) Carbohydrate-Associated Epitope-Based Anti-Cancer Drugs and Vaccines. *Advances Biosci Biotech* 4: 18-23.

41. Lee G, Huang C, Liu S, Zhang H (2013) The Immunology of Cancer Cells. *Symbiosis Open J Immunol* 1: 4-7.

42. Lee G, Huang C, Tang Y, Zhang H (2013) Potential Roles of Cancerous Immunoglobulins in the Immunology of Cancer Cells. *J Clinical Cell Immunology* 5: 200-206.

43. Lee G, Liu S (2013) Roles of Cancerous Antigen Receptors and Ca215 in the Innate Immunity of Cancer Cells. *Open J Immunol* 3: 127-138.

44. Lee G, Zhang H, Tang Y (2013) Anti-Gnrh Receptor Monoclonal Antibodies as Bioequivalent Analogs of Gnrh. In: Sills Es, Editor.: Nova Biomedical, ed. SaF Gonadotropin-Releasing Hormone (GnRH): Production 10: 157-174.

45. 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. [Crossref]

46. Hamm A, Veeck J, Bektas N, Wild P, 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. [Crossref]

47. Zamanian-Daryoush M, Lindner D, Tallant T, Wang Z, Buffa J, et al. (2013) The Cardioprotective Protein Apolipoprotein A1 Promotes Potent Anti-Tumorigenic Effects. *J Biol Chem* 288: 21237-52. [Crossref]

48. 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. [Crossref]

49. Huang Z, Ung T (2013) Effect of alpha-1-acid glycoprotein binding on pharmacokinetics and pharmacodynamics. *Curr Drug Metab* 14: 226-238. [Crossref]

50. Rolny C, Mazzone M, Tugues S, Laoui D, Johansson I, et al. (2011) Hrg Inhibits Tumor Growth and Metastasis by Inducing Macrophage Polarization and Vessel Normalization through Down regulation of Plgf. *Cancer Cell* 19: 31-44. [Crossref]

51. Lee G (2014) Widespread Expressions of Tcrs in Cancer Cells and the Implications in Cancer Immunology: T Cell Receptors (Tcr): Structure, Role in Immune Responses and Health Implications. Nova Publisher 56: 155-168.

52. Lee G, Huang C, Ge B (2014) Two Distinct Humanized Monoclonal Antibodies for Immunotherapy of Ovarian Cancer. *J Cancer Sci Ther* 6: 110-116.

53. 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. [Crossref]

54. Jiang D, Ge J2, Liao Q3, Ma J4, Liu Y5, et al. (2015) IgG and IgA with potential microbial-binding activity are expressed by normal human skin epidermal cells. *Int J Mol Sci* 16: 2574-2590. [Crossref]

55. Dieplinger H, Ankerst DP, Burges A, Lenhard M, Lingenhel A, et al. (2009) Afamin and apolipoprotein A-IV: novel protein markers for ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 18: 1127-1133. [Crossref]

56. Bruno R, Chaikin P, Berille J, Olivares R (2003) 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.

57. Lee G (2015) Dual Differential Roles of Cancerous Immunoglobulins as Suggested by Interactions with Human Serum Proteins. *J Clin Cellular Immunol* 6: 7.

58. Lee G (2015) Functional Roles of Cancerous Immunoglobulins and Potential Applications in Cancer Immunodiagnostics and Immunotherapy, in Innovative Immunology, AP Group, Editor. Khan WA: New Jersey.

59. 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. [Crossref]

60. Misra UK, Pizzo SV (2013) Evidence for a pro-proliferative feedback loop in prostate cancer: the role of Epac1 and COX-2-dependent pathways. *PLoS One* 8: e63150. [Crossref]

61. Misra UK, Pizzo SV (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. [Crossref]

62. López-Árias E, Aguilar-Lemarroy A, Jave-Suárez LF, 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. [Crossref]

63. Macuks R, Baidekalna I, Gritcina J, Avdejeva A (2010) Apolipoprotein A1 and Transferrin as Biomarkers in Ovarian Cancer Diagnostics. *Acta Chirurgica Latviensis* 10.

64. Krajewska E, Lewis C, Chen Y, 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. [Crossref]

65. 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. [Crossref]

66. Lee G (2015) Ca215, a New Pan Cancer Biomarker, and Its Clinical Applications. *Cancer Sci Res Open Access* 2: 1-6.

67. Lichenstein HS, Lyons DE, Wurfel MM, Johnson DA, McGinley MD, et al. (1994) Afamin is a new member of the albumin, alpha-fetoprotein, and vitamin D-binding

protein gene family. *J Biol Chem* 269: 18149-18154. [Crossref]

68. Fournier T, Medjoubi-N N, Porquet D (2000) Alpha-1-Acid Glycoprotein. Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology 1482: 157-171.

69. 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. [Crossref]

70. Jerkovic L, Voegele A, Chwatal S, Kronenberg F, et al. (2005) Afamin Is a Novel Human Vitamin E-Binding Glycoprotein Characterization and in Vitro Expression. *J Proteome Res* 4: 889-99. [Crossref]

71. Jones A, Hulett M, Parish CR (2005) Histidine-Rich Glycoprotein: A Novel Adaptor Protein in Plasma That Modulates the Immune, Vascular and Coagulation Systems. *Immunol Cell Biol* 83: 106-18. [Crossref]

72. 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. [Crossref]

73. Sehgal I, Forbes K, Webb M (2003) Reduced Secretion of Mmps, Plasminogen Activators and Timps from Prostate Cancer Cells Derived by Repeated Metastasis. *Anticancer Res* 23(1A): 3942. [Crossref]

74. Li Y, Rizvi S, Ranson M, Allen B (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. [Crossref]

75. 75. Lee G, Chow S, Chien C and Liu S (2015) Anti-Gnrh Receptor Monoclonal Antibodies, First-in-Class Gnrh Analog. *J Gynecol Res* 1: 102.

76. Lee G, Liu S (2015) Monoclonal antibodies against hepatitis B viral surface antigens and epitope grouping. *Monoclon Antib Immunodiagn Immunother* 34: 90-95. [Crossref]

77. Lee G, Liu S1 (2015) Monoclonal Antibodies Against Human Cardiac Troponin I for Immunoassays II. *Monoclon Antib Immunodiagn Immunother* 34: 169-173. [Crossref]

78. Vázquez J, González L, Merino A, Vizoso F (2000) Expression and clinical significance of apolipoprotein D in epithelial ovarian carcinomas. *Gynecol Oncol* 76: 340-347. [Crossref]

79. Müller M, Strand S, Hug H, Heinemann E-M, Walczak H, et al. (1997) Drug-Induced Apoptosis in Hepatoma Cells Is Mediated by the Cd95 (Apo-1/Fas) Receptor/Ligand System and Involves Activation of Wild-Type P53. *J Clin Invest* 99: 403-413. [Crossref]

80. Ganz PA, Baras M, Ma PY, Elashoff RM (1984) Monitoring the therapy of lung cancer with alpha-1-acid glycoprotein. *Cancer Res* 44: 5415-5421. [Crossref]

81. Liyan W, Yang S, Zhu P, Yu Y, et al. (2015) Tumor Vaccine Candidates Formed by Peptide Mimics of the Carbohydrate-Associated Epitopes on the Heavy Chain of Cancer Cell-Expressed Immunoglobulin. *Cancer Immunol Immunother*.

82. Lee G (2016) Ovarian Cancer Immunotherapy. Epitope/Peptide-Based Monoclonal Antibodies for Immunotherapy of Ovanrian Cancer. Vol. In press. 35.

83. 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. [Crossref]

84. 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. [Crossref]

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