Introduction

It is known for decades that immunoglobulins can be expressed by many cancer cells of epithelial origins for unidentified functional roles. RP215 was the first monoclonal antibody generated in 1987 and shown to react with a carbohydrate-associated epitope located mainly in the variable regions of heavy chains of immunoglobulins expressed by cancer cells (designated as CA215), but not in those of B cell origin. Through years of biological and immunological studies, it has become apparent that dual differential roles are played by cancerous immunoglobulins. Therefore, cancerous immunoglobulins are essential for the growth and protection of cancer cells under our body environment. RP215 was found to be a unique probe for CA215 in the immunoassays to monitor serum levels of shed cancerous immunoglobulins among cancer patients for immunodiagnostic applications. Upon binding with surface expressed immunoglobulins, RP215 was shown to induce apoptosis and complement-dependent cytotoxicity to many cancer cells. Humanized forms of RP215 can be used to target cancer cells of different tissue origins and are being developed into antibody-based anti-cancer drugs for cancer immunotherapy. A new generation of chimeric antigen (CAR)-T cell technology is being utilized to introduce humanized RP215 gene transfected to T cells for cancer immunotherapy of selected sets of human cancers.

Keywords: RP215, CA215, Anti-cancer drugs, Immunoglobulins, Chimeric antigen receptor (CAR).

Abstract

Parallel to the conventional immunology, immunoglobulins can also be produced by many cancer cells of epithelial origins for unidentified functional roles. RP215 was the first monoclonal antibody generated in 1987 and shown to react with a carbohydrate-associated epitope located mainly in the variable regions of heavy chains of immunoglobulins expressed by cancer cells (designated as CA215), but not in those of B cell origin. Through years of biological and immunological studies, it has become apparent that dual differential roles are played by cancerous immunoglobulins. Therefore, cancerous immunoglobulins are essential for the growth and protection of cancer cells under our body environment. RP215 was found to be a unique probe for CA215 in the immunoassays to monitor serum levels of shed cancerous immunoglobulins among cancer patients for immunodiagnostic applications. Upon binding with surface expressed immunoglobulins, RP215 was shown to induce apoptosis and complement-dependent cytotoxicity to many cancer cells. Humanized forms of RP215 can be used to target cancer cells of different tissue origins and are being developed into antibody-based anti-cancer drugs for cancer immunotherapy. A new generation of chimeric antigen (CAR)-T cell technology is being utilized to introduce humanized RP215 gene transfected to T cells for cancer immunotherapy of selected sets of human cancers.

Keywords: RP215, CA215, Anti-cancer drugs, Immunoglobulins, Chimeric antigen receptor (CAR).
may lose consciousness. Hyperventilation may occur in some donors as a result of anxiety [6].

Cancerous immunoglobulins, CA215 and RP215

RP215 is one of 8,000 monoclonal antibodies generated against a serous ovarian cancer cell line, OC-3-VGH [2]. The target antigen generally designated as CA215 was shown to be recognized by RP215 through of a unique carbohydrate-associated epitope located mainly on the immunoglobulin heavy chains expressed by cancer cells of epithelial origins, but not found in those produced by normal B cells. In addition to cancerous immunoglobulins (42%) and related immunoglobulins superfamily proteins which can account for as much as 60%, RP215-specific epitope was also identified in mucin or normal hyperplastic epithelial cells, and others not related to immunoglobulins such as mucin [1,2,4,6,10,23,24].

Glycol-analysis was performed to elucidate the carbohydrate-associated epitope recognized by RP215. Both CA215 and cancerous immunoglobulins (cIgG) consist of glycans of tri-saccharine (Galα1-3Galα1-4NeuAc) which are related to Core I O-glycan structures with 3-linked or 3,6-linked GalNAcitol, attached with the terminal N-acetyl neuraminic acid (NeuAc). In view of the high binding affinity between CA215 and RP215, it is assumed that the unique protein backbone structure of CA215 or immunoglobulin superfamily related proteins and tri-saccharide glycans are coordinated to constitute a unique RP215-specific epitope [4,6,25-30]. Similar observations with protein peptide-carbohydrate combinations were also found in the epitope recognition by selected anti-mucin 1 antibodies [4,31,32].

Effect of RP215 on Growth Inhibition of Cancer Cells in Vitro and in Vivo

Judging from the epitope nature of RP215, cancerous immunoglobulins can be recognized specifically by this antibody, similar to that of anti-human IgG. Therefore, RP215 can serve as the substitute to probe immunoglobulins and investigate their possible roles in biological functions among cancer cells [2,9,31,33]. Several cancer cell lines such as DU145 (prostate), A509 (lung), C33A (cervix) and OC-3-VGH (ovary), which show high expressions of immunoglobulins on cancer cell surface were employed to study induced apoptosis in the presence of RP215 or anti-human IgG by TUNEL apoptosis assay [3,34].

Generally speaking, RP215 and its humanized forms were shown to induce apoptosis to cultured cancer cells (OC-3-VGH ovarian cancer cells) in vitro, similar to that by anti-human IgG. In addition, CDC (complement-dependent cytotoxicity) reactions can also be induced by RP215 or anti-IgG. Typical results of such studies are presented in Figure 1 and Figure 2 for RP215, humanized RP215 and anti-human IgG, respectively in the concentrations range of 1 and 10 μg/ml. Furthermore, RP215 was also shown to significantly reduce the volumes of the implanted tumour (ovarian or lung cancer) in the presence of RP215, in a nude mice animal models [2,35,36]. Results of such studies are summarized and presented in Figure 3.
Figure 2: Complement-dependent cytotoxicity assay to demonstrate complement-dependent lysis of OC-3-VGH ovarian cancer cells with different treatments in the presence or absence of complement.

Negative controls include no treatment (control), complement only (C), normal human IgG plus complement (NH IgG+C), and normal mouse IgG plus complement (NM IgG+C), respectively. Treatments which resulted in a significant percentage of lysis included humanized RP215 plus complement (HRP215+C), murine RP215 plus complement (MRP215+C), and goat anti-human IgG plus complement (GAH IgG+C), respectively. The symbols of * and ** represent statistical significance of P<0.01 and P<0.001, respectively [37], (Use with permission).

Figure 3: Nude mouse experiments to demonstrate the dose-dependent effect of injected RP215 on implanted tumor volumes.

(A) Effects of injected RP215 in nude mice experiments with the OC-3-VGH ovarian cancer cell line as the model. On day 0, mice were inoculated with OC-3-VGH cancer cells. Mice (n = 4 for each group) were injected with either no antibody (negative control) or drug treatment (positive control, antibody high dose, or antibody low dose). Drug treatment with 60 mg/kg cyclophosphamide (positive control) or 10 mg/kg RP215 (antibody high dose) or 2 mg/kg RP215 (antibody low dose) were given to mice by intraperitoneal injection on the same day. Tumor volumes from mice of various treatments were determined on day 15. The symbols of * and ** represent statistical significance of P<0.05 and P<0.001, respectively [37,38]. (Use with permission.)

(B) Effects of injected RP215 in nude mice experiments with the SK-MES-1 squamous lung cancer cell as the model. 0.2 ml of cultured SK-MES-1 cancer cells were implanted at exponential growth phase into mice underneath the armpit or via the back (SK-MES-1 cell concentration: 1.5-2.5x10⁷ cells/mL). Three weeks after inoculation, the implanted tumors in each mouse become visible. Mice were then divided randomly into 4 groups (n = 5 for each group) and were injected with phosphate buffered saline (negative control) or drug treatment (positive control, antibody high dose, or antibody low dose). In the positive control group, mice were injected with 1000 μg/m² Genicitabine and cisplatin (80-100 mg/m²) in each dose. Both the antibody low dose and antibody high dose had mice injected with 0.14 mg/mouse/dose or 0.75 mg/mouse/dose, respectively, of RP215. In all 4 groups, antibody or drug treatments were performed twice in total during the 4th and 5th week following tumor cell implant. At the 6th week mark, the tumours were taken out and their volume measured. Tumor volumes were determined on the 6th week after tumor implant of SK-MES-1 cancer cells and antibody or drug injections with 5 mice for each treatment group. The symbols of * and ** represent statistical significance of P<0.05 and P<0.001, respectively [37,38], (Use with permission.)

Effect of RP215 Binding on Gene Regulations of Cancer Cells

Correlation Analysis of the Changes in Gene Expression Levels

The data represent the relative gene expression levels of TLR-3 (I), NFkB-B-1 (II), P21 (III), P1 (IV), IgG (V), c-fos (VI), cyclin D1 (VII), TLR-9 (VIII), and TLR-4 (IX) in the OC-3-VGH ovarian cancer cells following separate treatments with RP215 and anti-human IgG of the OC-3-VGH ovarian cancer cell line.

The correlation coefficient between these two anti-antigen receptor ligands was determined to be R²=0.9577 [8,37], (Use with permission).

Effect of RP215 binding on gene regulations of cultured cancer cells was investigated [39]. OC-3-VGH ovarian and C33A cervical cancer cell lines were used as models. From results of comparative
growth inhibition studies, it can be assumed that RP215 may behave functionally similar to anti-antigen receptors (eg. Anti-IgG or anti-T cell receptors) to all cancer cells when analyzed by semi-quantitative RT-PCR method; it was generally observed that genes involved in growth/proliferation of cancer cells are influenced parallelly upon treatments of cultured ovarian cancer or cervical cancer cell lines with either RP215 or anti-antigen receptors [2,11,34,40]. Among more than a dozen genes studied, upregulations of genes including NFKB-1, IgG, TCR (T cell receptor) and ribosomal proteins were found. This is in contrast to down regulations of genes related cell growth such as cyclic D1 and c-fos. In the case of Toll-like receptors (TLR), significant gene expression changes were observed upon similar treatments.

The results of this study are consistent with the potential roles of TLRs in carcinogenesis and growth/proliferation of cancer cells. For example, TLR-3 is significantly up-regulated, whereas TLR-6 and TLR-9 are down-regulated. High correlations were observed among gene expression changes of a number of TLRs and other growth related genes, when cancer cells were treated with RP215 or anti-human IgG. Results of expression changes of as many as ten genes, following treatments with RP215 and anti-IgG were compared parallelly through correlation analysis. As shown in Figure 4, excellent correlations of these two IgG ligands are obtained (R² = 0.9577) [2,21,25,41].

Dual Differential Roles of Cancerous Immunoglobulins in Cancer Cells

In an attempt of elucidate the actual functional roles of cancerous immunoglobulins, interactions between human serum proteins and cancerous immunoglobulins (clgG) were investigated by using affinity-purified CA215 and cancerous immunoglobulins. Human 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 between those purified by CA215 and by clgG affinity columns, respectively. Generally speaking, they can be classified with either pro-cancer or anti-cancer properties [12,42]. Among the known pro-cancer human serum proteins which can be recognized by CA215 and/or clgG are C4 binding proteins α-chain, complement C3 and complement factor H, serotransferrin and vitronectin etc. [14,24,43-48].

For the captured serum anti-cancer proteins, the following were identified: Apolipoprotein A1, fibrinogen β-chain, inter-α-trypsin inhibitor heavy chain 4, anastellin, keratin type 1 cytoskeletal 9 and antoimmune IgG. These observations led us to believe that two differential roles of cancerous immunoglobulins exist in the unique immune system of all cancer cells, in vitro and in vivo [26,37,49-51]. Cancerous immunoglobulins can serve as specific binding proteins to capture some serum proteins to promote growth of cancer cells [12,42,52,53]. At the same time, they can also neutralize or interact those with anti-cancer properties in human circulations [26,54-56]. The modes of interactions between human serum components and cancerous immunoglobulins on the cancer cell surface are currently unknown and remain to be explored in the future [57-59].

Immunodiagnostic Application of RP215 for Monitoring Serum CA215 Levels among Cancer Patients

In view of the fact that CA215 contains multiple epitope recognized by RP215, this monoclonal antibody can serve simultaneously both for capturing and signal detection of CA215 in a sandwich enzyme immunoassay for the monitoring of serum CA215 levels among cancer patients [17,18].

RP215-based enzyme immunoassay was employed to analyze serum levels of CA215 for ovarian and cervical cancer patients. It was generally observed that elevated serum levels of CA215 are cancer stage-dependent in either type of cancer [2]. It was clearly demonstrated that positive detection rates of CA215 are correlated well with cancer diagnosis and stages [18,60]. In the case of ovarian cancer, the positive rates range from 58-86% depending on clinical stages when compared to normal individual. For cervical cancer patients, the positive rates were as high as 66-94% at various cancer stages [61,62]. Stage-dependence of serum CA215 levels was analyzed with cases of either ovarian or cervical cancer, especially those at Stage I vs Stage II and Stage III are clearly differentiated. Based on these experimental observations, we believe that CA215 enzyme immunoassay kit can be beneficial for routine monitoring of patients with cervical or ovarian cancers at different stages [2].

In a separate study, serum CA215 levels of given patients were monitored at different treatment time intervals or period of medical treatments for cervical or ovarian cancer [19,20,63]. The results suggest that serum levels of CA215 seem to correlate well with the tumor size or burden of a given patient [61,30]. Seven days after surgical treatments or chemotherapy, the CA215 levels were found to decrease significantly compared to those without medical treatments. Therefore, routine monitoring of cancer patients with serum CA215 should be beneficial to those patients undergoing medical treatments [2,18,60].

A large scale evaluation of serum CA215 levels was performed for more than 500 specimens from patients confirmed with different cancers [38,62,64]. Based on the results of RP215-based enzyme immunoassays, positive detection rates of 50% or higher were found in serum specimens of cancer patients who have lymphoma (83%), or cancer of the lung (52%), liver (74%), esophagus (61%), stomach (60%), ovary (59%), breast (71%) or cervix (51%). The same set of serum specimens was also used for quantitative determinations of other known cancer biomarkers such as CA215, CA15-3, CA19-9, AFP, CEA, Cyfra21-1 and β2 micro globulins [33,65,66]. When CA215 was combined with other types of cancer biomarkers for cancer detection, much higher positive rates were generally observed than those markers used singly. For example, in the case of ovarian cancer, the combined positive detection rates for both CA215 and CA125 increase from 59% to 82%, when clinical studies were performed in three separate major medical centers [2,31,32]. Results of such studies are summarized in Table 1.
Table 1: Comparative positive detection rates of various cancers by CA215-based and other cancer-associated antigen-based enzyme immunoassay kits [17,37].

<table>
<thead>
<tr>
<th>CA215 (0.1Au/mL)</th>
<th>Lung (n)</th>
<th>Liver (n)</th>
<th>Ovary (n)</th>
<th>Esophagus (n)</th>
<th>Breast (n)</th>
<th>Stomach (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA (5 ng/ml)</td>
<td>I 52% (112)</td>
<td>74% (58)</td>
<td>61% (23)</td>
<td>71% (44)</td>
<td>60% (30)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II 67% (33)</td>
<td>54% (35)</td>
<td>47% (19)</td>
<td>95% (20)</td>
<td>50% (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III 94%</td>
<td>81%</td>
<td>-</td>
<td>65%</td>
<td>96%</td>
<td>70%</td>
</tr>
<tr>
<td>AFP (20 ng/ml)</td>
<td>I 74% (58)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II 50% (40)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III -</td>
<td>85%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CA125 (35 Au/ml)</td>
<td>I 52% (112)</td>
<td>74% (58)</td>
<td>59% (68)</td>
<td>61% (23)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II 85% (13)</td>
<td>85% (13)</td>
<td>59% (66)</td>
<td>50% (12)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III 85%</td>
<td>92%</td>
<td>82%</td>
<td>75%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CA19-9 (37 Au/ml)</td>
<td>I 74% (58)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>II 55% (22)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III -</td>
<td>82%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CA15-3 (30 Au/ml)</td>
<td>I -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>II -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III -</td>
<td>82%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B2 microglobulin (2.6 ng/ml)</td>
<td>I 74% (58)</td>
<td>59% (68)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>II 56% (16)</td>
<td>90% (10)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III -</td>
<td>81%</td>
<td>100%</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyfra21-1 (3.3 ng/ml)</td>
<td>I 52% (112)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>II 50% (52)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III 77%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a: the concentration in each bracket ( ) is the cut-off value for each respective cancer biomarker; b: n refers to the number of patient cases; c: I: CA215 only; d: II: other marker only; e: III: combined, modified taken from [19] with permission.

Cancerous Immunoglobulin’s and Immunotherapeutic Applications

Surface-expressed Cancerous Immunoglobulins as Target for RP215-Based Anti-Cancer Drugs

Since the discovery of RP215 monoclonal antibody in 1987, it has become a unique probe for functional studies of immunoglobulins expressed by cancer cells and their potential clinical applications [7,39,67]. Specific binding interactions of the surface expressed immunoglobulins with RP215 or its humanized forms can result in growth inhibition of cancer cells in vitro and in vivo [9,39]. Therefore, surface expressed cancerous immunoglobulins can become a unique target for RP215-based anti-cancer drugs. Based on this assumption, RP215 could be developed for potential immunotherapeutic applications.

Basically, two strategies are currently being used for the development of RP215-based anti-cancer drugs. First of all, RP215 which is of mouse origin should be humanized through appropriate modification by genetic engineering work [9,32]. The heavy chain Fc region of IgG should be replaced by human one (preferably with human IgG1, Fc sequences). The Fab regions can be modified into human forms, (FR1 to FR4), except with the minimum alteration of CDRs antigen binding domains (CDR1 to CDR3) through computerized modelling [48,68,69]. Similar work was made with light chain modification. The humanized antibodies of RP215 may be assembled in different forms including whole antibodies, fragments such as Fab, Fab’ and F(ab’)2 as well as recombinantly produced single chain antibodies (scFv), which are immunoreactive to CA215. The resulting humanized forms should be of equivalent or comparable affinity and specificity to the original murine RP215 and usually contain substantially similar or identical CDR regions or domains [70-72].

The substantial bioequivalence between humanized forms and murine RP215 was demonstrated by Sandwich and/or binding immunoassays [32,42]. Furthermore, comparative biological functional assays, such as induced apoptosis and CDC reactions were also performed [12,35]. Based on these criteria, bioequivalence between humanized RP215 and murine RP215 was established and qualified for further preclinical and clinical studies [2]. Stable CHO cell lines secreting humanized RP215 are being developed by CRO services to produce gram scale of designated humanized RP215 by in vitro culture. Preclinical studies by using humanized RP215 are being carried out to meet the criteria of IND as required by US FDA. Infusion of gram quantity of humanized RP215 would be required to demonstrate the anti-cancer efficacy in cancer immunotherapy.
Application of RP215-related CAR-T Technology in Cancer Immunotherapy

DNA sequences in the variable domains of heavy and light chains of humanized RP215 are re-engineered into the single chain variable regions fragments (scFv) [39,73,74]. scFv are inserted into a suitable vector together with activation signal genes derived from T-cells. Following transfection to T cell, chimeric antigen receptors (CAR) containing scFv of RP215 can be expressed in modified T cells. The entire process involves extracting a patient’s T cells, and then transfecting CAR inserted T cells back to the same patient. This would result in the in vivo expression of RP215-related scFv gene which might target cancerous immunoglobulins on cancer cell surface. RP215-related CAR-T system may represent a promising approach of cancer immunotherapy to those with high expression of cancerous immunoglobulins on the surface of respective targeted cancer cells [2,16,37,75-83].

Conclusions

Through decades of investigation, the mechanisms of action of RP215 monoclonal antibody as a candidate of anti-cancer drugs have been elucidated. The target antigen designated as CA215 was found to consist mainly of immunoglobulin heavy chains expressed by most of human cancer cells of epithelial origin. The epitope is located mainly on the variable regions of cancerous immunoglobulin heavy chains, but not in those of normal B cells [2,62].

Similar to goat anti-human IgG, RP215 can serve to target immunoglobulins expressed on cancer cell surface. RP215 could result in the induced apoptosis and CDC reactions to cancer cells in vitro [2,62]. With nude mouse animal models for proof of concept, injections of RP215 could also reduce the volume of the implanted tumor (ovarian and lung cancer cell lines models) [11,65,84]. Furthermore, humanized RP215 is available for further preclinical and clinical studies. Based on the experimental observations, we believe that humanized RP215 can be a suitable candidate for anti-cancer drugs for patients with multiple indications of human cancer.

Therefore, in this review, we have highlighted the humanized forms of RP215 for potential anti-cancer drugs development in the future. Either by passive immunization or CAR-T cell therapy, most of human cancer can be targeted to achieve effective immunotherapy.

References


