

RP215 Monoclonal Antibody-Based Anti-Cancer Vaccines

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How to cite this paper: Lee, G. (2026)
RP215 Monoclonal Antibody-Based Anti-
Cancer Vaccines. *Advances in Bioscience
and Biotechnology*, 17, 512-521.
<https://doi.org/10.4236/abb.2026.179030>

Received: August 24, 2026

Accepted: September 26, 2026

Published: September 29, 2026

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Abstract

RP215 is a monoclonal antibody generated against an ovarian cancer cell extract. RP215 was shown to react specifically with a carbohydrate-associated epitope located mainly in the variable regions or Fab regions of heavy chain immunoglobulins designated in general as CA215. This unique and specific epitope was shown to be expressed by almost all cancer cells in humans, but not detected in normal immunoglobulins of B cell origins. Through extensive studies during the last two decades, RP215 was shown to be a good cancer biomarker which reveals strong inhibitions to many human cancer cells *in vitro* or *in vivo*. RP215-specific epitope was found to be immunodominant, since only RP215 epitope-related monoclonal antibodies were generated upon immunization of mice with RP215-affinity purified CA215 as the immunogens. Therefore, rat anti-idiotypic (anti-id or Ab2) monoclonal antibodies were generated against RP215-idiotypic regions and shown to bear the internal images of RP215-specific epitope. Following immunizations of mice with purified rat anti-id (Ab2), the induced Ab3 sera revealed similar immunological and biological properties to those of original RP215. Therefore, anti-id (Ab2) in this case may serve as immunogens or vaccines to elicit strong Ab3 which mimic passively immunized RP215 in many anti-cancer properties. It can be suggested that anti-id monoclonal antibodies can be suitable candidates for development of anti-cancer vaccines which may have potential clinical applications in therapeutic or preventive treatments of human cancer.

Keywords

RP215/CA215, Anti-Idiotypic (Anti-Id), Cancer Vaccines, Ab2/Ab3

1. Introduction

RP215 is one of the three thousand monoclonal antibodies generated against an OC-3-VGH ovarian cancer cell line [1]. This antibody was shown to react specifi-

cally with a carbohydrate associated epitope in the variable regions of cancer cell-expressed immunoglobulin heavy chains designated in general as CA215 [1] [2]. However, this unique specific epitope is not detected in normal human immunoglobulins [3]. RP215 was shown to inhibit the growth of tumor cells of many human tissue origins through many *in vitro* and *in vivo* studies during the last two decades [4]. Since both carbohydrate moiety and amino acid residues are involved in the unique epitope recognized by RP215, attempts were made to demonstrate its immunodominance and elucidate its likely primary structures through comprehensive complex carbohydrate analyses [5].

Alternatively, one can actively immunize humans with anti-idiotypic (anti-id or Ab2) antibodies of RP215 to elicit Ab3 responses. Similar to RP215, the circulating Ab3 sera can serve as substitute of passively immunized RP215 to inhibit or eliminate cancer cells among the immunized individuals. Therefore, the immunodominance of the RP215-specific epitope needs to be demonstrated through the generation of anti-id antibodies and critically assessed for the developments of anti-id-based anti-cancer vaccines for applications in cancer therapy. Experimental evidences were highlighted to reveal the structural characteristics of RP215-specific epitope and to demonstrate its immunodominance, through generation of monoclonal antibodies against immun-affinity purified CA215.

Through the generation of rat anti-id monoclonal antibodies (Ab2), the bioequivalence between RP215 and subsequent Ab3 upon active immunizations with rat anti-id (Ab2) could be demonstrated. Comprehensive biological and immunological analyses of the resulting Ab2 should provide a logical basis for using anti-id antibodies (Ab2) of RP215 for application of anti-cancer vaccine in humans.

2. Structural Basis for Immunodominance of Rp215-Specific—Epitope

2.1. Elucidation of Molecular Structures of RP215-Specific Epitope

Early studies revealed that RP215 reacts with an epitope that is sensitive to treatment with NaIO₄ and/or protease [1] [6]. When cancer cells were cultured in a defined serum-free medium in the absence of sugar precursors, the RP215-specific epitope was not detected in the recovered shed culture medium by using typical RP215-based EIA assays for quantitation of normal CA215 [7]. Therefore, it is assumed that RP215-specific epitope is structurally dependent on the presence of carbohydrate. Further comprehensive glyco-analysis was performed in collaboration with NIH Complex Carbohydrate Research Center (CCRC) [8] to elucidate the O-linked glycan structure which is part of specific RP215 epitope. The exact carbohydrate-associated primary structure of RP215-specific epitope was hypothetically assigned. As shown in **Figure 1(b)**, the O-linked glycan is part of epitope formation in CA215.

Upon treatment of culturing cancer cells with tunicamycin to inhibit biosynthesis of N-glycans, the binding activity to CA215 was not affected [6] as shown in **Figure 1(c)**. Therefore, it can be assumed that N-glycan may not be involved in the epitope formation of RP215-specific epitope in CA215.

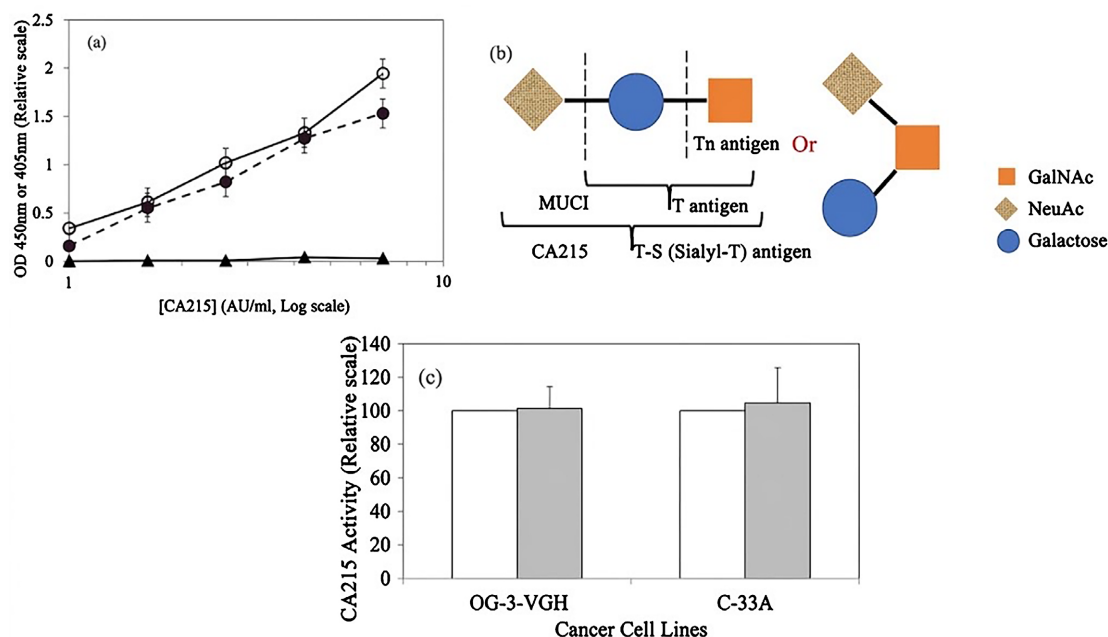


Figure 1. (a) RP215-based enzyme immunoassays to reveal dose-dependent signal or activity of CA215 (○) expressed in AU/mL and effects of goat antihuman IgG-Fc (1 µg/ml) (●) as well as goat antihuman IgG-Fab (1 µg/ml) (▲) on CA215 signal or activity; (b) Elucidated and proposed O-glycan structure associated with RP-215-specific epitope; (c) Effects of 48 hr tunicamycin treatments (1 µg/mL) on CA215 activity in the supernatant of two cultured cancer cells for OC-3-VGH and C-33A.

By using RP215 coated wells and purified CA215 as the antigen for competitive enzyme immunoassay [6], three human IgG domain-specific detection probes were used for competitively binding studies, including goat anti-human IgG-Fab (alkaline phosphatase-labeled), goat antihuman IgG-Fc (alkaline phosphatase-labeled) and RP215 (horse-radish peroxidase-labeled). As shown in **Figure 1(a)**, goat anti-human IgG-Fc probe has little effect on CA215 binding signal. However, with goat anti-human IgG-Fab as the probe, CA215 signals were totally inhibited as clearly demonstrated. Based on the results of these comparative studies, it can be concluded that RP215-specific epitope is located at the Fab or variable regions expressed by cancerous heavy chain immunoglobulins, but not in Fc domains of human IgG.

As shown in **Figure 1(b)**, the O-linked glycan structure which appears to be associated with RP215-specific epitope is a Core 1 structure with 3-linked GalNAcitol and/or 3,6-linked GalNAcitol. The identified O-linked glycan appears to be sialyl T antigen (Ts antigen) which appears to be associated with many types of cancer cells in humans. This may be caused by aberrant termination of O-glycan biosynthesis and attachment to cancerous heavy chain immunoglobulins.

2.2. Demonstration of Immunodominance of RP215-Specific Epitope

The uniqueness of RP215-specific epitope lies on the fact that both the carbohydrate moiety and the amino acid residues are involved in the epitope formation of RP215. Attempts were made to use RP215-affinity purified CA215 as immunogens

to generate additional monoclonal antibodies in mice. Unexpectedly, the only monoclonal antibodies (or hybridomas) recovered were those highly related to RP215 including RCA100, RCA10, RCA105, RCA110 and RCA111. They were grouped into three sub-epitope groups, all of which are highly specific to CA215, although with distinct peptide/nucleotide sequences, and affinity constants to CA215.

RCA100 and RP215 were found to have identical peptide and nucleotide sequences. However, common binding properties are shared by the list of RP215 and other newly generated RCA antibodies, including: (1) loss of CA215 binding upon antigen treatment with 100 mM NaIO₄, (2) RP215 binding to CA215 can be 100% inhibited by any of RCA antibodies; and (3) any of the RCA antibodies can pair with RP215-HRP in sandwich immunoassays for CA215 quantitation. Results of these comparative analyses are summarized in **Table 1**. The results of these studies indicated that RP215-specific epitope are commonly shared by these anti-CA215 monoclonal antibodies, although distinct structural differences are found.

By using dot blot assay, only RP215 and other five RCA antibodies showed positive bindings with CA215 isolated from cancer cells. When Western blot assay under either denatured or renatured conditions, only RP215, RCA10 and RCA100 showed positive staining with CA215, whereas the others showed no binding signal under the same conditions. The observations would indicate that the latter may react with sensitive conformational structures of the RP215 epitope.

Table 1. Amino acid and nucleotide homology among RP215, RCA-10, RCA-100, RCA-104, RCA110 and RCA-111.

Group	Mab	Immunogen	% homology of Amino Acid Sequence	Neucleotide Homology	Estimated Kd (nM)	Degenerate Primer Pairs ^b				CA215 Binding ^d
						PDI→PDI	PDI→PDI	PDI→PDI	PDI→PDI	
Group I	RP215	OC-3-VGH Cancer Cell Extract	100%	100%	~4	+	+	+	+	+
	RCA-100	CA215 ^c	100%	100%	~4	+	+	+	+	+
	RCA-10	CA215 ^c	98%	99%	~4	–	+	+	+	+
Group II	RCA-104	CA215 ^c	65%	84%	~4.5	–	+	+	+	+
	RCA-111	CA215 ^c	65%	84%	~4.5	–	+	+	+	+
Group III	RCA-110	CA215 ^c	65%	84%	~0.35	–	+	+	–	+
Control	GHR106	GnRH receptor	46%	N.A. ^a	N.A	N.A	N.A	N.A	N.A	N.A

^aNot available, ^bDegenerate primer; sets used: P_{D1}: 5'-ACTAGTCGACATGAAATGCAGCTGGGTCATSTTCTTC-3'; P_{D11}: 5'-ACTAGTCGACATGGGATGGAGCTR TATCATSYTCTT-3'; P_{D111}: 5'-ACTAGTCGACATGGGATGGAGCKGGA-WCTTTCWCTT-3'; P_{Drev}: 5'-ACTACCCGGGYCTCCACACACAGGRRCCAGTGGATAGAC-3'. P_L: 5'-ACTAGTCG-ACATGGGGWTCAAGAT-3'; P_{krev}: 5'-ACTACCCGGGTGGATGGTGGGAAG-3'; R: A or G; K: G or T; Y: C or T; W: A or T; ^cImmunogen, CA215 is affinity purified; ^dCommon CA215 binding properties are shared by the listed Mabs including: (1) Lost of CA215 binding upon antigen treatment with 100 mM NaIO₄ overnight; (2) RP215 binding to CA215 can be totally inhibited by any of RCA Mabs. (3) Any of RCA Mabs can pair with RP215-HRP in sandwich EIA for CA215 quantitation.

The TUNEL assay was performed to compare relative degrees of induced apoptosis of culturing cancer cells by RP215 and these RCA antibodies. Results of such comparative studies are presented in **Figure 2**. Under the concentration of 1 $\mu\text{g}/\text{mL}$ for each antibody, the cancer cells were cultured separately for 48 hours, the increase in apoptosis was assessed. Significant increase in apoptosis was detected in each of the CA215-specific monoclonal antibodies. Furthermore, complement-dependent cytotoxicity (CDC) assay was also performed in the presence of rabbit complements, and 10 $\mu\text{g}/\text{mL}$ each of these anti-CA215 monoclonal antibodies. All of these antibodies showed positive CDC reactions under the normal culture conditions.

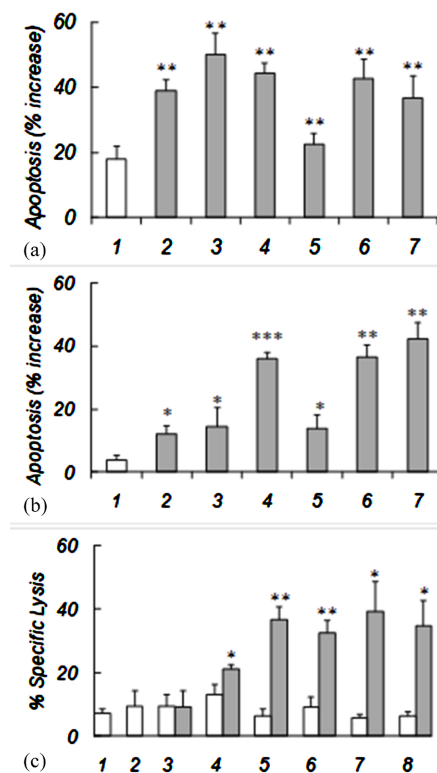


Figure 2. (a) The apoptosis (TUNEL) assay to demonstrate the increase in apoptosis of cancer cells in response to the incubation of OC-3-VGH cancer cells for 48 h with the following antibodies (10 $\mu\text{g}/\text{mL}$ for each): Lanes 1 - 7 correspond to that of normal mouse IgG, RCA-10, RCA-100, RCA-104, RCA-110, RCA-111, and RP215, respectively. (b) The apoptosis assay (TUNEL) at the Mab concentration of 1 $\mu\text{g}/\text{mL}$ to demonstrate the apoptosis of cancer cells in response to the treatment of OC-3-VGH cancer cells for 48 h with the following antibodies: Lanes 1 - 7 correspond to that of normal mouse IgG, RCA-10, RCA-100, RCA-104, RCA-110, RCA-111, and RP215, respectively. The statistical significance is indicated by (* $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.005$). (c) The complement-dependent cytotoxicity assay to demonstrate the induction of cell lysis of OC-3-VGH cancer cells by various RCA Mabs at 10 $\mu\text{g}/\text{mL}$ in the presence of complement (3 $\mu\text{L}/\text{test}$) as demonstrated by the following: Lane 1: no treatment; Lane 2: freshly prepared rabbit complement; Lane 3: mouse IgG (white column) and mouse IgG plus complement (grey column); Lane 4: RCA-10 (white) and RCA-10 plus complement (grey); Lane 5: RCA-100 (white) and RCA-100 plus complement (grey); Lane 6: RCA-104 (white) and RCA-104 plus complement (grey); Lane 7: RCA-110 (white) and RCA-110 plus complement (grey); Lane 8: RCA-111 (white) and RCA-111 plus complement (grey).

Based on the results of such investigations, we believe that RP215-specific epitope may consist of the combination of both carbohydrate and peptide moiety which would result in a strong immunodominance. This assumption can be judged from the high recovery of the subsequently generated RCA monoclonal antibodies upon immunizations with purified CA215 in mice. The observations reported in this study would certainly support the generations of anti-id antibodies (Ab2) which may mimic the internal images of RP215-specific epitope. These Ab2 antibodies can be further characterized and used as immunogens or vaccines to actively immunize individuals to produce strong Ab3 responses. The circulating Ab3 sera may be biosimilar to the infused RP215 for therapeutic applications in treatments of human cancer. A typical example of such investigation can be demonstrated as described in the following section.

3. Anti-Idiotypic Antibodies of RP215 and RCA(s) for Anti-Cancer Vaccine Developments

3.1. Generations and Characterizations of Anti-RP215-id and Anti-RCA-ID(s) Monoclonal Antibodies [Ab2(s)]

Initially, F(ab')₂ peptide fragments of RP215 which contain Ab1 or RP215-id sequence were isolated and used as immunogens to generate monoclonal antibodies in rats. Monoclonal antibodies specific to RP215-id were selected and shown to carry internal images of RP215-specific epitope by comparative binding assays to the well-coated RP215.

This was demonstrated in **Figure 3(a)**. By using one of rat anti-RP215-id monoclonal antibodies designated as R26J17. It was clearly demonstrated that both CA215 and this Ab2 antibody in appropriate dilutions showed dose-dependent bindings to well-coated RP215. Furthermore, R26J17 antibody was shown to serve as Ab2 and surrogate to CA215 in a sandwich EIA by using RP215 for both coating and capturing antibodies as presented in **Figure 3(b)**.

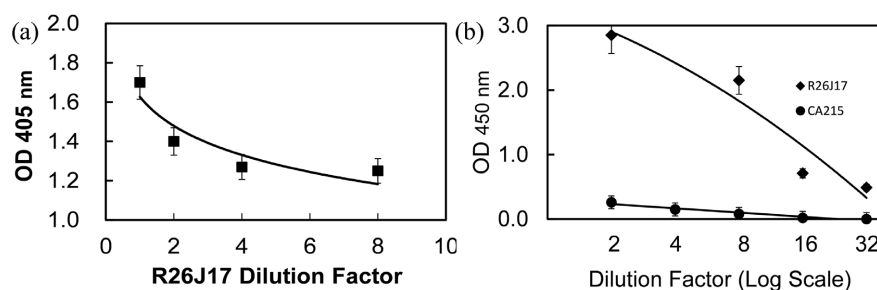


Figure 3. (a) A typical binding assay was performed to demonstrate the binding of purified Ab2 (R26J17) monoclonal antibody to RP215-id (or Fab) coated on microwells. Goat anti-rat IgG labelled with alkaline phosphates was used as the detecting antibody in this assay. Initial antibody concentration was 1 µg/mL; (b) Ab2 (R26J17, Anti-RP215-id) antibody from the cell culture supernatant was used as surrogate of CA215 in a sandwich EIA by using RP215 for both well coating and enzyme conjugate in a 60 min assay. Initial antibody and CA215 concentration was 1 µg/mL. Results reveal the biochemical equivalence or similarity between Ab2 and CA215.

3.2. Vaccinations of Ab2 to Induce Ab3 Immune Responses for Anti-Cancer Vaccine Development

Recombinant R26J17 (RecR26J17) was mass-produced and used as immunogens (Ab2) to induce Ab3 responses in mice as the model. Ab3 sera were characterized with respect to their ability to serve as the surrogate of RP215. This was demonstrated by binding assays with CA215-coated wells. Ab3 sera from three immunized mice at 1:500 dilutions were found to have binding activities to well-coated CA215 comparable or better than those of RP215. The results of such comparative studies are presented in **Figure 4**.

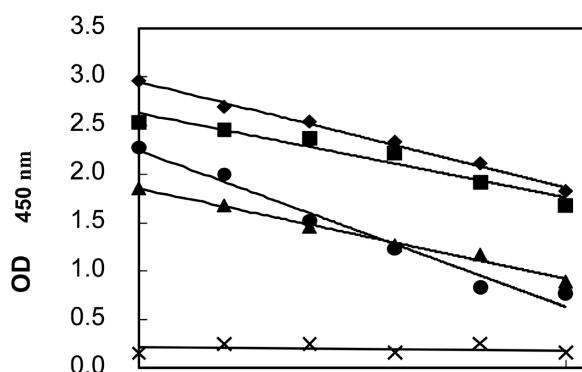


Figure 4. Comparative binding assays of Ab3 antisera (anti-R26J17) and RP215 (Ab1) to CA215 coated wells. Ab3 antisera were obtained from three mice which were actively immunized with Ab2 (R26J17 monoclonal antibody) and used initially with 1:500 dilutions. For RP215 initial concentration of 1 µg/mL was used. Normal mouse serum of the same dilution was used as the negative control. Symbols of the figure: (▲) Ab3-I; (●) Ab3-II; (■) Ab3-III; (○) RP215; and (x) negative control, normal mouse serum.

3.3. Evaluations and Characterizations of Ab3 Antisera as a Surrogate of RP215 in Anti-Cancer Vaccine Development

By immunohistochemical studies, both RP215 and Ab3 sera were shown to stain positively and comparably to as many as twenty different permanent human cancer cell lines as summarized in **Table 2**. By TUNEL apoptosis assays, it was clearly demonstrated that RP215 and Ab3 sera inhibit the growth inhibition of cancer cells in culture such as OC-3-VGH ovarian cancer cells and C33-A cervical cancer cells. The comparative assay results are presented in **Figure 5**. The concentrations of CA215-specific Ab3 immune sera are equivalent to be around 1 – 2 mg/ml of RP215. Western blot assay also revealed that RP215 and Ab3 sera stain identical protein bands of 50 - 60 KDa corresponding to those of heavy chain immunoglobulins expressed on the surface of cancer cells. Based on these comparative studies, it can be ascertained that RP215 and Ab3 are biosimilar in terms of their respective biological and immunological properties. Furthermore, rat anti-RP215-id monoclonal antibodies as Ab2 or their respective mRNA formats can be mass-produced and formulated to serve as anti-id-based vaccines in humans. The elicited Ab3 immune response upon vaccinations of immunized individuals with R26J17

(Ab2's) may mimic the immunological and biological properties of RP215 for applications in anti-cancer therapy.

Table 2. Immunohistochemical staining results of various cancer cell lines with RP215 and other relevant antibodies.

Cancer Types	Cell Lines	ATCC Number	RP215	Ab3 ^a	COX-100 ^b
Brain	Neura2A		±*	±	ND
	SH-SY5Y	CRL-2266	+	+	+
	U-87MG	HTB-14	+	+	ND
Breast	MCF7	HTB-22	±	±	ND
	MDA-MB-231	HTB-26	+	+	ND
	MDA-435		+	+	ND
	T-47D	HTB-133	±	±	ND
Cervical	C-33A	HTB-31	+	+	ND
	Hela	CCL-2	–	–	+
	ME-180	HTB-33	+	+	ND
Colon	HCT 115		+	+	+
	HCT 116	CCL-247	+	+	+
	HT-29	HTB-38	+	+	ND
	HT-441		+	+	+
	SW-48	CCL-231	+	±	+
Kidney	FS293		+	+	ND
Leukemia	K562	CCL-243	–	–	+
Liver	Hep-2		+	+	ND
	HepG2	HB-8065	±	±	ND
	Hep3B	HB-8064	+	+	ND
Lung	A549	CCL-185	+	+	ND
	Calu-6	HTB-56	+	+	+
	MRC-5	CCL-171	+	+	ND
	WI-38	CCL-75	+	+	+
Lymphoma	HEL		–	–	+
Melanoma	MMAN		+	+	ND
	SK-Mel-3	HTB-69	–	–	+
Ovary	OC-3-VGH		+	+	ND
	OVCAR-3	HTB-161	+	+	ND
	SK-OV-3	HTB-77	+	+	ND
Placenta	BeWo	CCL-98	±	±	ND
Prostate	DU145	HTB-81	±	±	ND
	PC-3	CRL-1435	±	±	ND

^aAb3: Antisera raised rat anti-RP215-idiotype monoclonal antibody. ^bCOX-100: Anti-human IgG-Fc monoclonal antibody. *Staining intensities follow the order of – (negative), ± (borderline) and + (positive). ND: not determined.

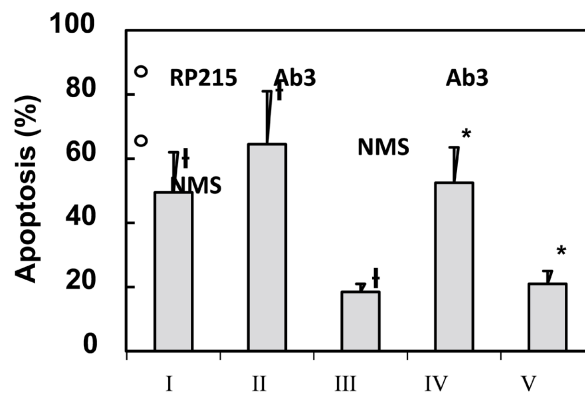


Figure 5. Percentage increases in apoptosis of cancer cells after incubation with RP215 (10 µg/mL) and Ab3 antisera (1:500 dilution) in TUNEL assay, Lanes I, II, and III are percentage increases in OC-3-VGH ovarian cancer cell apoptosis after 24 hr incubation with RP215, Ab3 antisera and normal mouse serum of the same dilution (negative control), respectively. Lane IV and V are C-33A cervical cancer cell apoptosis after 72 hr incubation with Ab3 and normal mouse serum of the same serum dilution.

4. Conclusion

In this short review, anti-tumor activities of Ab3 immune sera originally derived from anti-id of RP215 were clearly demonstrated and can be used for treatments of many types of human cancer. Rat anti-id monoclonal antibodies such as R26J17 employed in this investigation appear to be a suitable candidate for cancer vaccine developments, provided strong Ab3 immune responses can be elicited in immunized individuals. This may be the rational basis of using anti-id of RP215 for anti-cancer vaccine developments in various vaccine formulations. However, generated A2 and A3 responses in terms of variations in affinity and specificity to human cancer may be predicted as well instead of passive immunizations with large amount of original RP215 monoclonal antibody, Aab2's can serve as the surrogate for active immunizations in humans for therapeutic and preventive application in treatments of human cancer.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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