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Idiotype Antibody Network Regarding Malignant Cell Regression in the Brain Tumor Patients Treated with the Natural Human Monoclonal Antibody, Pritumumab

Abstract

Pritumumab is a natural human monoclonal antibody derived from a lymph node of a patient with cervical carcinoma. The recognized antigen is an altered form of vimentin called, ecto-domain vimentin (EDV) that is expressed on the cell surface of cancer cells. EDV is present on vimentin-exposing ectosomes (VEE) that are vesicular protrusions on the cell surface of cancer cells. Idio-33 is an anti-paratactic idiotype antibody of pritumumab that recognizes a p34Ag from glioma cells. During a Phase II clinical trial with brain tumor patients, Idio-33 was used to monitor serum levels of pritumumab (Ab1). In the treated patients, the paratope of pritumumab behaved as a mimotope of p34Ag that induced the expression of anti-anti-idiotype antibody (Ab3). This Ab3 showed a rhythmic response like Lorenz chaotic attractor in good responders and not in poorly responding patients. The involvement of EDV and VEE in tumor immunity is complex but yet dynamic and may be involved in tumor stem cell regression.

Keywords: Pritumumab; Anti-Cancer Idiotype Antibody; Human Monoclonal Antibody; Ecto-Domain Vimentin; Vimentin-Exposing Ectosome; Tumor-Associated Antigen; Fractal Physiology; Lorenz Chaotic Attractor

Introduction

Pritumumab (also known as CLN-IgG or CLN-H11 or ACA-11) is a natural human IgG1 monoclonal antibody derived from a regional draining lymph node of a patient with cervical carcinoma [1,2]. Pritumumab was used in a Phase II clinical trial for brain tumor patients in Japan during 1991 – 1996 and efficacy results including complete responses (CR) and partial responses (PR) were about 9-fold better than standard therapy with little side effects [3]. In this review we reassess anti-tumor mechanism underlying pritumumab active immunotherapy by shedding a light on antigenicity of vimentin and its ligand p34. Both antigen/epitopes are recognized by idiotype antibody networks through an interface of human tumor immunity.

Background

When does a normal cell become a malignant cell? How does a malignant cell regress? Identifying the steps may suggest ways to eradicate malignant cells. The answer to this question may depend on what kind of molecule is involved in the normalization of cancer stem cells by means of an appropriate signal transduction pathway responding to the tumor cell microenvironment (T-niche). Carcinogenesis is a gradual process through several steps with multi mutational changes of the cell [4]. Tumor cell promotion is related to chronic inflammation [5]. These theses rise the other question whether repairing mutational sites and the strategy of totally killing tumor cells lead to curing malignancy. Currently, it is known that cancer is caused by normal stem cells accompanied by the communication with a T-niche that involves tumor cells, fibroblasts, neural cells, endothelial cells, immune effector cells and stromal cells, which is at the core of the cancer stem cell hypothesis [6]. To identify cancer stem cell is the most relevant research in cancer cell biology so that the molecular markers distinct differences between normal stem cells and cancer stem cells (cancer initiating cells) has been extensively studied. If there is an outstanding difference designating malignancy, the host immune response should be able to recognize the difference and these tumor antigens would be quickly eliminated. Even one amino acid alteration in tumor antigen can be recognized by host immune surveillance. Therefore the altered tumor antigen presents only subtle difference to tumor immunity. Are there any mechanisms via immune response to help cancer patients to recover from malignancy? Eradication of malignant cells may depend on how cancer stem cells can revert to normal stem cells [7]. This means that tumor cells develop reciprocally through both states of cancer stem cell and normal stem cell [8].

Drug Discovery

In our drug discovery program utilizing the intelligence of the natural human immune response we focused on the draining lymph node of the tumor where the host immune systems were sensitized by tumor cells [7-9]. It is reasonable that host immune surveillance was not only defensive but also offensive for epithelial homeostasis. To probe the natural human anti-cancer immune response we initially developed human x human hybridoma technology to immortalize the B-lymphocyte and isolated a human monoclonal antibody (human mAb) that reacts with tumor cells [1,2]. We successfully cloned a human x human hybridoma termed CLN-H11 secreting a human monoclonal antibody IgG (CLN-IgG; pritumumab) that recognized the autologous tumor cell antigen that is called Ecto-Domain Vimentin (EDV) [10,11]. This altered vimentin is expressed both in the cytoplasm and the cell surface [12]. Especially, EDV is present on Vimentin-Exposing Ectosomes (VEE) that are vesicular protrusions on the cell surface of tumor cells [13].

Clinical Responses

GMP manufactured pritumumab was used in several Phase II clinical trials with brain cancer patients in Japan. Based on the summarized results [3,14] it was determined that pritumumab was an effective anti-cancer mAb for brain tumor patients. During the clinical trials patient’s serum samples were analyzed for the presence of both Ab1 (pritumumab) and Ab3 responses by use of anti-paratactic idiotype antibody (Ab2) termed with Idio-33. The correlation between responders and those who developed an Ab3 response was significant and highly correlated with patient recovery. Furthermore, the singular circaseptan rhythm of the paratope internal image of pritumumab, using Idio-33, was observed in the serum of patients and showed Lorenz-like chaotic attractor suggesting this may be a good prognostic indicator of how brain tumor patients may respond [15].

Efficacy of Pritumumab toward Malignant Gliomas

The overall responses of the malignant brain tumor patients with repetitive administration of pritumumab through Phase I [16], early phase II [17] and late phase II [18] was summarized in 2009 [3]. The
Overall response rate was about 28%. The protocol was planned with 1 mg twice a week through systematic intravenous administration for the duration of 6 months. Tumor progression was monitored with magnetic resonance imaging (MRI). Shown in Figure 1 is a Complete Response (CR: 100% shrinkage tumor mass, PR: >50%, MR: >25%, NC: no change, PD: progressive disease) from a pritumumab treated patient. In addition to obtaining MRI data for each patient careful attention was paid to the neurological changes during each phase since brain tumors directly affect the symptoms in regarding to the central nervous system. In summary, the efficacy with CR + PR was about 28% and CR + PR + MR + NC about 70% with neither remarkable side effects nor recurrence [3,14]. With some patients an Ommaya reservoir was used for targeting tumors directly with pritumumab, however since tight fibration hampered successive administration of pritumumab so that systemic intravenous administration was employed. Since the half-life of pritumumab in the serum was 75 hrs the intravenous administration twice a week was reasonable. According to FDA guidelines (“Points to consider in the manufacture and testing of monoclonal antibody products for human use 1987”) the amount of DNA contained in a single injection of biotechnologically produced drugs should be 10pg or less. Pritumumab was purified (purity >98%) using Protein-A affinity chromatography from serum free cell cultures of the original human x human hybridoma CLN-H11 (19-21). With this culture system was a challenge to have the human DNA levels less than 10pg/injection in the final preparation, which limited the mAb injections to no more than 2mg. A dose of less than 0.5 mg/injection was not feasible since the measurement of serum clearance even with high sensitive ELISA was not possible. In addition, a 2 mg/injection dose made it difficult for the clinician to continue the repetitive administration due to the narclocalep symptom of the patient. Therefore a 1 mg/injection dose twice a week was safely used.

Clinical grade pritumumab was prepared in the GMP facility of Japan Pharmaceutical Development Co., under mandated quality control with validated procedures with respect to specificity, purity, stability, solubility, biological activity, toxicity, pharmacokinetics, productivity and showing negative mycoplasma and human virus contamination, including CJV (Creutzfeldt-Jakob Prion). The permeability of BBB (Blood Brain Barrier) and accessibility of pritumumab toward brain tumor solely were evaluated during the course of medical development by use of pritumumab’s paratope specific anti-idiotypic antibody, Idio-33 [13].

Anticancer Antibody Pritumumab (Ab1) And Paratactic Idiotypic Antibody Idio-33 (Ab2) Presenting Its Counter Paratope

Pritumumab is a naturally fully human IgG1 (γ-heavy chain, κ-light chain) secreted from human x human hybridoma CLN-H11 (CLN-SUZ-H11) having human type glycosyl chain generated by the use of the draining cerebral lymph node lymphocytes (CLN) of the adenosquamous cell carcinoma of the uterus cervix of a Japanese woman (SUZ). At the time of diagnosis the patient had completely healthy constitution with no addiction to cigarettes, alcohol, or drugs. Patient SUZ had a hysterectomy and an uterectomy including the removal of a wide range of regional lymph nodes. In histochemical observation, patient SUZ uterus auxiliary lymph nodes were sensitized and formed giant follicular lymphocytes that expanded in the germinal centers, and which were palpable on physical examination. However no autoimmune symptoms were observed except for the tumor cells that metastasized in the lymph nodes and lungs. The fusion partner was the lymphblastoid B-cell line UT729-6 [1,2]. The amino acid sequence of pritumumab was determined by the DNA cloning of both the heavy and light chains from the original CLN-H11 hybridoma. The tertiary structure of the antigen combining site of pritumumab was constructed by the use of the computer assisted analysis BIOCE program [15] (CLN-F(ab’), model in Figure 2). The idiotypic antibody that recognizes the antigen binding site of pritumumab, the paratactic anti-idiotypic antibody Idio-33 (Ab2), was generated by a murine hybridoma that was made by fusing the lymphocytes immunized by CLN-IgG F(ab) fragments and the murine myeloma, NS1. The total amino acid sequence of Idio-33 was also determined by DNA cloning, and the tertiary structure of antigen combining site was constructed by the BIOCE program (Idio-33-\text{V}_{\text{p34}} model in Figure 2). The paratope or epitope signature of the complementarity determining regions (CDR’s) on the tertiary structure of an antibody is called idiotope standing for a posture of the internal chaperonic p34 via idiotypic antibody network [15]. The immune surveillance recognizes the subtle abnormal change of the integrity of the networks woven by the intermediate filament proteins under oncogenic stress. The specificity of Idio-33 to the paratope of CLN-IgG is useful for dosage determination and follows serum clearance, immunohistochemical distribution, accessibility, and permeability of pritumumab [15].

**Biological activity of Pritumumab and its Counter Paratope Regarding Tumor Cell Traits**

It is understood that the binding activity of an Ab to its Ag does not always elicit tumor suppressive activity. In some cases, the Ab stimulates tumor growth. Autoimmunity, which appears in Hashimoto disease, shows a risk of carcinogenesis [23]. A monoclonal antibody against EGF receptors show tumor promotion activity in some cases [24]. Therefore it is worthwhile to evaluated whether the pritumumab obtained from the sensitized lymph node lymphocytes of the cerebral...
Antigen combining site of the antibody (Ab1), an idiotype, is constituted by the six complementary determining regions (CDR’s) expressing on the consolidated tertiary structure of the variable region of the heavy chain (VH) and the variable region of the light chain (VL). A certain idiotype is determined as an antigen recognized by anti-idiotype antibody (Ab2) which is called an idiotope. The paratope of anti-idiotypic antibody (Ab2) behaves as an antigen, called paratopic idiotypic Ab2, toward anti-anti-idiotypic antibody (Ab3). Likewise Ab1→Ab2→Ab3→Ab4→Ab5 recognitions subsequently transmits (idiotope image transmission) until the cessation of a certain immune response toward a pathogenic antigen. Each idiotypic antibody maneuvers idiotypic Ab networks cooperating with immune effector cells including T-cell, B-cell, MΦ, CD’s, reactive astrocyte, microglia, and so on in the brain. The CLN-IgG-F(ab’)_2 paratope (shown yellow color on left side) is a counter image to Idio-33-V_L (shown red color on right side). They carry convex-concave internal images on the CDR’s and they dock each other with a special affinity constant. We can envision the CLN-IgG-F(ab’)_2 paratope resembles the p34Ag epitope and the Idio-33-V_L presents the EDV. The space filling models of each Ab was constructed in silico by the BIOCE program (NEC) by use of the amino acid sequences determined from the cloned DNA sequences of mAb from human hybridoma CLN-H11 and murine hybridoma Idio-33.

**Figure 2:** Idiotope recognition between CLN-IgG and Idio-33 mimics the ligand p34Ag epitope and vimentin antigen/epitope in respective antigen combining site constituted by CDRs.

cancer patient has tumor suppressive activity. A cervical cancer cell line was inoculated on the back of athymic mice and the effect of the antibody was tested by the peritoneal injection of pritumumab with various binding activities to the antigen/epitope. The tumor growth was suppressed according to the activity of pritumumab (Figure 3a). This tumor suppressive activity of pritumumab was in good accordance with the affinity of pritumumab toward the idiotope of Idio-33 (Figure 3b). Statistical analysis of the correlation coefficient revealed a significant correlation (p<0.001) between the affinity of pritumumab to Idio-33 and the tumor regression as measured by the reduction of tumor mass-grafted on the back of athymic mice. This result showed that denatured pritumumab may change the structure of its paratope resulting in a decrease of its binding affinity to the epitope of Idio-33. Thus, denatured pritumumab should no longer effectively suppress the tumor growth after losing the affinity to EDV. Other data showed pritumumab recognized CD133³ brain tumor stem cells. From the cancer stem cell hypothesis, malignancy associated tumor cell traits such as metastasis, invasiveness, loss of contact inhibition, and chemotherropy resistance are proved by the minor cancer stem cell population but not by the major non-stem cancer cells or differentiated cancer stem cells [25]. Cancer stem cells in brain tumors are generated from normal stem cells in a certain tumor niche [26]. Therefore, a scheme of tumor generation can be envisioned by analyzing aberrant epigenetic programming and/or signal transduction pathways in cancer stem cells [27]. From the dichotomous aspect of cancer stem cell Hugwil AV proposed the potential praxis of reprogramming cancer stem cells to their normal condition by the active homeostasis of tumor immune response against the altered vimentin [8]. Particularly regarding the tumor suppressive activity of pritumumab, the dynamic interaction between the cytoskeletal intermediate filamentous protein vimentin and its ligand p34 is the major concern about the mechanism of tumor cell regression from the malignant state.

**Vimentin**

Vimentin is a hallmark of Epithelial-Mesenchymal Transition (EMT) and its counter phenomenon Mesenchymal-Epithelial Transition (MET) of tumor cells. These cancer stem cell transformations are deeply linked to tumor cell characteristics, such as malignant traits;

1) Cell surface vimentin is a cancer stem cell marker associated with malignancy [28].
2) Highly malignant tumor cells were positive for vimentin via 14-3-3εs over expression [29].
3) Vimentin is a scaffold protein in invadosomes of the invasive cancer cells [30].
4) Vimentin forms with Hsp90 complex in geldanamycin induced apoptosis [31].
5) Vimentin-beclin-14-3-3 complex participates in the regulation of autophagy [32].
6) Inhibition of vimentin expression attenuates wound healing [33].
7) Peptides involved in tumor angiogenesis bind to vimentin [34].
8) Vimentin is the ligand to Dectin-1 as an innate immunity receptor [35].
9) Vimentin cooperates with NOD2 in conjunction with innate immunity [36].
10) Over expression of vimentin is relating to tumor heterogeneity via cellular coalescence [37].

These tumor traits are reflections of vimentin modification, modulation and alteration by specialized enzymatic catalysis with;

a) citrullination at arginine residue regarding autoimmune recognition by T-cell [38].

b) palmitoylation of cytoskeleton associated protein regarding anti-proliferative signaling [39].

c) phosphorylation regarding the state of mitotic furrow conjunction with cytokinesis [40] and
d) sumoylation regarding cell migration of glioma [41].

Particularly stress response of vimentin networks with actin and tubulin assemblies elicits dynamic cytoskeletal integrative signaling between the cellular plasma membrane and nucleus to adapt to quick microenvironmental changes for maintaining epithelial homeostasis. An EVD is a posture of the tumor antigen/epitope residing on C2 domain of the region for vimentin intermolecular dissociation/association of filament formation (fasciculation) [42]. In this regard the interaction between EVD and p34Ag are quite relevant in fasciculation of vimentin and networking. Taken together with these vimentin relating cellular responses, it could be considered that the vimentin network behaves as a hub and spoke for the various kind of regulators, cofactors, modulator molecules, and chaperonic molecules which vimentin sequesters and expels to manipulate the stress responses of critical factors even in tumorogenesis. In the malignant cell, EVD was recognized by pritumumab on the special vesicular protrusions of the...
In order to examine the tumor suppressive activity of pritumumab, ME180 cervical carcinoma cells expressing the same A24 HLA type of the lymph node lymphocytes of the original patient were grafted subcutaneously on the back of athymic mice and then pritumumab showing various activities were injected intraperitoneally (in Figure 3a). The variation of pritumumab activity was reconstructed by the mixture of denatured pritumumab and 100% active pritumumab to Idio-33, measured as Idio-33 coated micro-titer plate and tested by ELISA. Before inoculation, 5 x 10^6 target tumor cells were mixed once with pritumumab at various activities. They showed no cytotoxicity to the target tumor cell in vitro. Length and width of the grafted tumors were measured every 2 to 5 days and the tumor sizes were determined by the equation: (length) x (width)²/2. The degree of affinity of pritumumab to Idio-33 reflected the degree of the affinity of pritumumab to EDV. In order to determine the correlation between the tumor suppressive activity and the binding activity of pritumumab to Idio-33, a correlation diagram (in Figure 3b) was made with the horizontal axis indicating indices determined by the sizes of the transplanted tumor and the vertical axis indicating indices determined from the binding activity of pritumumab to Idio-33 by ELISA.

**Figure 3:** Correlation between tumor suppressive activity of pritumumab and its affinity to the paratope of Idio-33.

The non-linear but a rhythmic Ab3 augmentation was analyzed by fractal analysis. The time series of the Ab3 concentration was plotted against itself using a time delay of 7 days (modes in Figure 4). The Ab3 waves observed in the serum of the good responders formed chaotic attractor as seen in Lorenz like chaotic orbit. This chaotic attractor did not form when the time delay was set at some other days except 7 days. The attractors have two fan blade shaped regions in which orbits spiral out from two fixed points centering the convergence of the antigen/epitope (upper three modes in Figure 4b). Noticeably, the first attractor transformed into the other attractor in the good responders in contrast we generated the secondary human x human hybridoma by use of peripheral lymphocytes of a brain tumor patient who responded well.

The Ab3 augmentation might be caused by Ab1 administration as the vaccine effect by the idiotypic antibody through the adjuvant effect of VEEs. An attempt was made to measure the inhibitory activity of the patient serum on the ability of pritumumab to bind to the target U251MG tumor cells but the serum did not show any hampering activity. Since the Ab3 augmentation was not be impeded by any other inhibitory immune responses, it seems to be caused by antigen specific B-cell networks. The antigen/epitope of vimentin was recognized by pritumumab on the outside of the plasma membrane of the VEE and VEEs contributed the formation of idiotypic antibody network through VEE-mediated idiotope image transmission.
Brain tumor patients were received 1 mg pritumumab twice a week for the duration indicated in horizontal axes of Fig. 4a. The concentration of pritumumab in the patient’s serum was measured intermittently two times in a week by use of anti paratactic idiotypic Idio-33 Ab. The concentration of Ab3 was measured from the amount of IgG corresponding to that of pritumumab calculated from the dose-dependent curve in ELISA with the Idio-33 coated micro-titer plates in the presence of 2M NaCl+5M Urea. The aperiodic augmentation of Ab3 was observed in the cases of CR, PR, and MR patient’s sera (left graphs Figure 4a). No augmentation was observed in the cases of PD patients (right graphs in Figure 4a). These aperiodic waves were studied in the time series analysis by use of Wolfram Mathematica program with plotting Ab3 titer at the points (X(i(n),X(i(n+2))) where i(n+2)−i(n)>7 days/time delayed. i(n): the date of the nth data point. X(i(n)): the concentration of Ab3 on i(n). The internal image of Ab1-paratactic idiotope augmented as indicated in Ab3 waves in Figure 4a were projected into an orbit resembles the Lorenz’s chaotic attractor. A fractal attractor was not observed in the case of non-responders (bottom in Figure 4b).

Figure 4: Chaotic attractor for the paratope image of anti-anti-idiotypic antibody (Ab3) in the serum of malignant glioma patients who showed good response to the repetitive administration with pritumumab (Ab1).

According to this new paradigm for the reprogramming cancer stem cells, some praxis to reverse cancer stem cell to normal stem cell have been approached. Hsa-miR520d induced hepatoma cells to normal liver tissue via a stemness-mediated process [55]. The induced neural stem cells (hNSC) were a part of an efficacious therapeutic strategy for glioblastoma brain cancer [56]. Activation of protein kinase PKA led to MET and loss of tumor-initiating ability [57].

Taking into account tumor cell traits manifested by subtle structural variation of vimentin via VEE-mediated horizontal propagation like a ripple effect on malignancy, to restrain the formation of tumor derived microvesicles and destress the aberrant distortion on vimentin fascicles by pritumumab has the potential praxia to regulate cancer stem cell propagation and stimulate the switch that tends to reprogram cancer stem cell (tumor initiating cell) to normal stem cell. Together with appropriate normalized T-niche cells facilitating active homeostasis of the human immune surveillance and neuronal surveillance may countervail malignant cell traits to normal organogenesis.

Conclusion and Perspective

Clinical trials of pritumumab to gliomas was effective and beneficial for brain tumor patients. Those who effectively responded to the EDV specific active immunotherapy evoked anti-anti-idiotypic antibody (Ab3) response and showed circaseptan rhythmic augmentation. The measurement of the patterns of idiotope image transmission of Ab3 could be applied for diagnosis, prognosis and prophylaxis of the cancer patient undergoing pritumumab active immunotherapy. The mechanism of tumor regression observed in glioma patients is still largely unknown. However the first signpost suggests that the mechanism involves the biochemical interaction between EDV and its ligand p34Ag. Besides EDV presentation on the vimentin-exposing ectosome (VEE) of the normal one under the appropriate microenvironment (N-niche) [8].
malignant cell is a synthetic mechanism for tumor cell promotion to provide a better understand how to regulate carcinogenesis.

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Conflict of Interest

Authors have no conflict of interest concerning this article.

References


