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Serum B-cell maturation antigen (BCMA) reduces binding of anti-BCMA antibody to multiple myeloma cells

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ABSTRACT

B-cell maturation antigen (BCMA), a tumor necrosis factor receptor (TNFR) family member, is selectively expressed on terminally differentiated B-lymphocytes including multiple myeloma (MM) tumor cells. We sought to determine whether circulating (c)BCMA in MM serum interferes with antiBCMA antibody binding to MM cells. An enzyme-linked immunosorbent assay (ELISA) was used to determine serum (s) BCMA levels among 379 samples from patients with relapsed/refractory MM (RRMM). Furthermore, flow cytometric and immunofluorescent studies were used to examine if concentrations of BCMA in patients' serum were high enough to interfere with the binding of anti-BCMA antibody to MM tumor cells. We have shown that BCMA is elevated in the serum from MM patients and that the median concentration of sBCMA from RRMM patients was 176 ng/mL (n = 379). Additionally, there was a consistent decrease in the binding of anti-BCMA antibody to MM tumor cells with sBCMA level ≥156 ng/mL. Together, these results demonstrate that circulating BCMA levels in most RRMM patients are high enough to interfere with anti-BCMA antibody binding to MM tumor cells and may interfere with BCMA-targeted immune-based therapies.

1. Introduction

Despite recent improvements in anti-MM therapy, nearly all patients develop resistance to their treatments [1–3]. Thus, finding therapies that better target MM cells is necessary to improve outcomes for these patients [4,5].

BCMA, a TNFR family member, is selectively expressed on terminally differentiated Blymphocytes including MM tumor cells [6–8]. BCMA is elevated in MM patient serum [9], and its levels correlate with their clinical status and survival [10]. γ-secretase, a multisubunit, intramembranous protein complex, is responsible for shedding BCMA from plasma cells [11]. γsecretase inhibitors prevent its shedding from MM cells [12] which may improve the anti-MM effects of BCMA-targeted therapies. Because BCMA is highly expressed on myeloma tumor cells and not on other non-Blymphocytes, this TNFR has become a common target in development of new anti-MM therapies [13]. Thus, those patients with high sBCMA most likely will have more BCMA on their plasma cells making them better candidates for such targeted therapies. However, recent studies have also shown that BCMA can exist in a free form, circulating BCMA. Thus, those patients with high levels of circulating BCMA may derive less clinical benefit from such therapies, because these soluble proteins may bind to the targeted antibodies preventing them from attaching on their intended target, the malignant plasma cell. Therefore, circulating BCMA may limit the efficacy of antibody-based therapies [4,5,14]. Thus, preventing its shedding off MM cells may be one way to improve the clinical activity of these immune targeted therapies.

In this study, we determined whether BCMA in MM serum and recombinant (r)BCMA interferes with anti-BCMA antibody binding to MM cells.

2. Materials and methods

2.1. Sample collection

Patients and healthy subjects provided informed consent in

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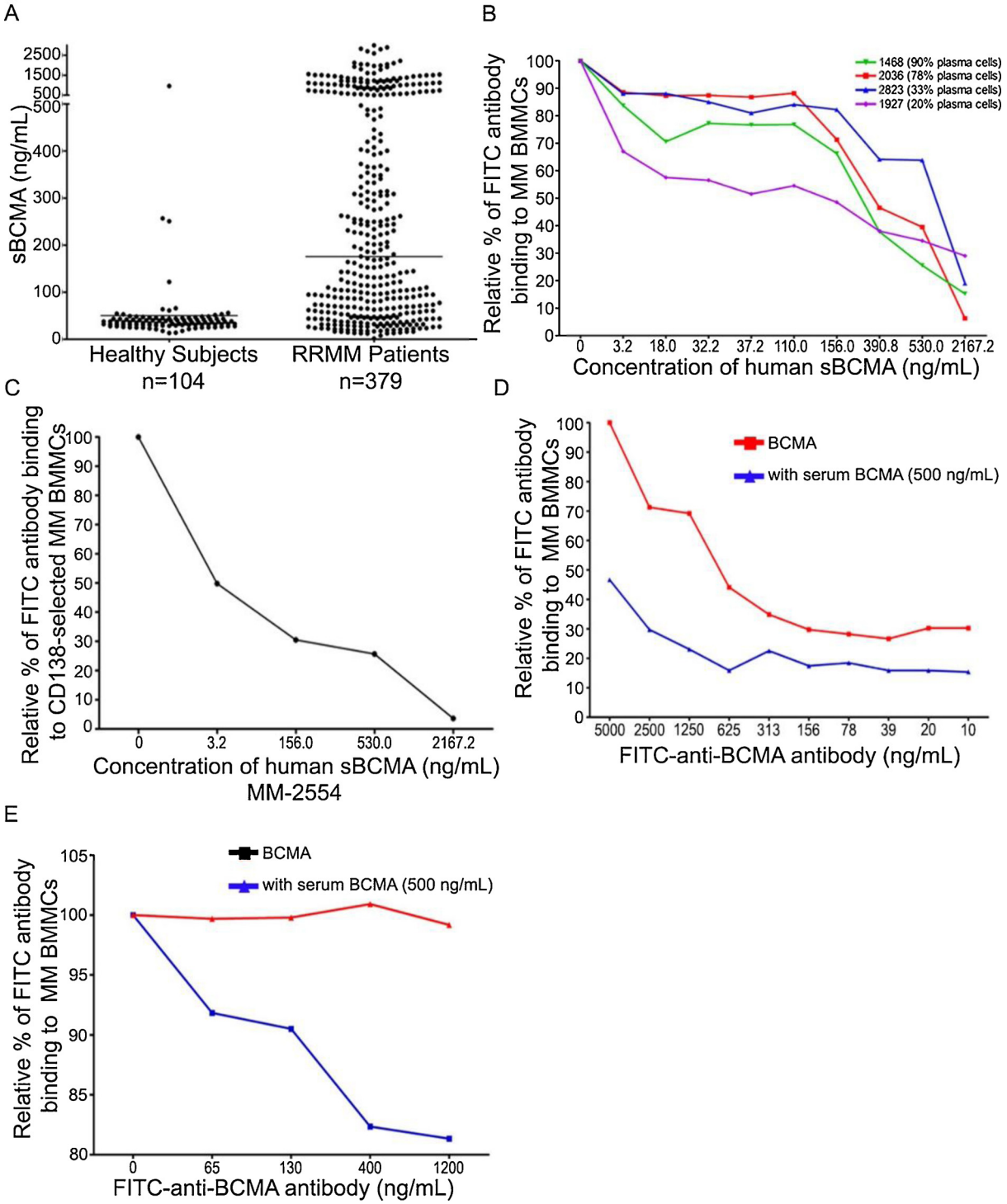

Fig. 1. (A) Serum BCMA levels are elevated in RRMM patients. Specifically, 379 RRMM patients have significantly higher sBCMA levels (median, 176.0 ng/mL; range, 1.8 to 4,865 ng/mL) than 104 age-matched healthy donors (median, 36.0 ng/mL; range, 13.4–958.1 ng/mL, $P < 0.0001$. (B) Determination of relative reduction in FITC-labeled anti-BCMA antibody (5,000 ng/mL) binding to MM BMMCs (MM Patient # 1468: Plasma cells 90%; 2036: 78%; 2823: 33%; and 1927: 20%) from MM serum with varying sBCMA concentrations (0–2,167.2 ng/mL). (C) Determination of relative reduction in FITC-labeled anti-BCMA antibody (5,000 ng/mL) binding to CD138- enriched MM BMMCs from MM serum with varying concentrations of sBCMA (0, 32.2, 156.0, 530.0, and 2,167.2 ng/mL). (D) Determination of the effects of MM serum containing sBCMA at 500.0 ng/ml sBCMA on FITC-labeled anti BCMA antibody binding (0–5,000 ng/mL) to MM BMMCs from MM Patient # 2554. (E) Evaluation of the effect of recombinant BCMA at concentrations from 0 to 1,200 ng/mL on FITC-labeled anti BCMA antibody (5,000 ng/mL) binding to MM BMMCs from MM Patient # 2816. Recombinant rhIgE-IgG-Fc protein was used as a negative control for FITC-labeled anti-BCMA antibody binding to MM BMMCs.

accordance with local institutional review board requirements and the Declaration of Helsinki (Western IRB BIO 001). Serum was collected and stored at −80 °C. MM bone marrow (BM) aspirates were obtained and mononuclear cells (MCs) were isolated. The human CD138 Magnet Selection kit (STEMCELL Technologies, Vancouver, BC, Canada) was used to enrich for plasma cells. The percentage of tumor cells were determined using anti-CD138 staining [15].

2.2. Assessment of sBCMA

Frozen MM patient serum samples were thawed and diluted 1:500. An enzyme-linked immunosorbent assay (ELISA) was used to determine sBCMA levels according to the manufacturer's protocol (R&D Systems, Minneapolis, MN, USA; catalogue # DY193E) and as we previously published was used to determine serum (s)BCMA levels [9,10]. The ELISA plates were analyzed using a μQuant (Biotek Industries, Winooski, VT, USA) plate reader set to 450 nm with KC Junior software. Each level represents the average of triplicate samples. It is important to note that this BCMA ELISA kit does not show cross reactivity with recombinant human a proliferation inducing ligand (APRIL), B-cell activating factor (BAFF), or recombinant human transmembrane activator and calcium modulator and cyclophylin ligand interactor (TACI)/Fc.

2.3. Flow cytometric analysis of anti-BCMA antibody binding to MM cells

One hundred μL of diluent buffer (1% BSA in PBS, pH 7.2–7.4, 0.2 μm filtered; R&D Systems, catalog #DY995) and MM serum were mixed with 50 μL of human (h) BCMA fluorescein-conjugated polyclonal antibody (R&D Systems, catalog # FAB193 F) to a final test concentration of 5 μg/mL. Five μg /mL is a clinically relevant concentration of BCMA that was detected in the serum of MM patients receiving antiBCMA antibody treatment [16]. Specifically, we utilized final tested concentrations of sBCMA at 3.2, 18.0, 32.2, 37.2, 110.0, 156.0, 390.8, 530.0, and 2,167.2 ng/mL and rBCMA (R&D Systems, catalog #193-BC-050) concentrations at 65, 130, 400, and 1200 ng/mL.

After one hour of incubation, samples containing the mixture of the anti-BCMA antibody and serum were added to fresh MM BMMCs. Cells were incubated for two hours and washed three times with wash buffer (PBS with 2% FBS, pH 7.2–7.4; Boston BioProducts, catalog #BM-222). Flow cytometric analysis was performed using a Beckman Coulter Cytomics FC 500 with Cytomics CXP software.

hBCMA fluorescein-conjugated polyclonal antibody was also used to determine sBCMA effects at a fixed concentration (500 ng/mL) on antibody binding to MM BMMCs. Fifty μL of the antibody was diluted with 50 μL of diluent buffer so that the final antibody concentrations were 1, 2, 39, 78, 156, 313, 625, 1250, 2,500, and 5,000 ng/mL. A control group was tested using anti-BCMA antibody (R&D Systems, catalog #AF193) added to primary MM BMMCs with 100 μL of diluent buffer without serum. The experimental group also included 100 μL of MM patient serum so that the final tested sBCMA concentration was 500 ng/mL. Samples containing the mixture of the anti-BCMA antibody and serum were incubated for one hour. They were then added to MM BMMCs which were incubated for two hours, and then washed. Flow cytometric analysis was performed using Beckman Coulter Cytomics FC 500 with Cytomics CXP software. The percentage change in hBCMA fluorescein-conjugated polyclonal antibody binding to MM tumor cells was determined by calculating the relative reduction in the proportion of MM BMMCs between those incubated with rBCMA or serum from MM patients compared with buffer alone.

2.4. Immunofluorescent analysis of anti-BCMA antibody binding to MM cells

Fifty μL of human BCMA fluorescein-conjugated polyclonal antibody was incubated for one hour with 100 μL of diluent buffer and 100 μL of serum from RRMM patients with final tested sBCMA concentrations of 300 and 1,000 ng/mL or with 100 μL of diluent buffer, and the mixture was added to MM BMMCs. Cells were incubated with anti-CD138PE (BD Bioscience, San Jose, CA, USA catalogue #552026) and anti-BCMA-FITC antibody (R&D Systems, catalog #FAB193 F) at a final concentration of 5,000 ng/mL (34 nM) overnight, washed and cytospun onto glass slides. 4', 6' diamino-2-phenylindole-2 HCl (DAPI) was added as a nuclear stain marker. The slides were washed and mounted with aqueous mounting media (Biomeda). BCMA protein and CD138 staining was visualized with a microscope and analyzed using the Microsuite Biological Suite program (Olympus BX51).

3. Results

3.1. sBCMA levels are elevated in RRMM patients

The level of sBCMA among 379 samples from patients with RRMM was higher (median, 176.0 ng/mL; range, 1.8 to 4,865.0 ng/mL; Fig. 1A) compared to 104 age-matched healthy donors (median, 36.0 ng/mL; range, 13.4–958.1 ng/mL; $P < 0.0001$).

3.2. sBCMA reduces anti-BCMA antibody binding to MM tumor cells

BMMCs from four MM patients with 90%, 78%, 33%, and 20% plasma cells were incubated with and without MM patient serum and FITClabeled anti-BCMA antibody. The concentration of FITC-labeled anti-BCMA antibody used was 5 μg/mL (34 nM; Fig. 1B), a concentration that falls within the range of 0.1–10 μg/mL that has been shown to have antibody-dependent cell-mediated cytotoxicity against tumor cells obtained from MM patients [16]. Reduced antibody binding was consistently observed once MM serum with nearly equimolar concentrations of sBCMA (26 nM [156 ng/mL]) were mixed with anti-BCMA antibody at 34 nM (5,000 ng/mL) in BMMC samples from all four MM patients with greater reductions in antibody binding at higher sBCMA concentrations, including decreases of 36%, 54%, 62%, and 62% when mixed with serum containing a final sBCMA concentration of 390.8 ng/mL and 71, 81, 85, and 94% at a final sBCMA concentration of 2,167.2 ng/mL in these four MM BMMC samples (Fig. 1B). Using CD138selected MM BMMCs, a similar sBCMA concentration dependent reduction in binding of anti-BCMA antibody to MM cells was observed (Fig. 1C). Anti-BCMA antibody concentrations from 105,000 ng/mL were incubated without or with a MM patient's sera so that the final BCMA concentration was 500 ng/mL (Fig. 1D). MM BMMCs in the presence of the highest tested concentration of anti-BCMA fluoresceinconjugated antibody (5,000 ng/ml [34 nM]) without serum demonstrated that antibody was bound to all the malignant cells (20% of the total cells in a patient whose BMMCs showed 20% plasma cell involvement) whereas in the presence of the MM serum containing BCMA at 500 ng/mL, the binding was reduced 55% (to 9% of cells; Fig. 1D). Although the percentage of tumor cells bound by antibody decreased when cells were exposed to lower concentrations of antibody, there was a similar percentage reduction in antibody binding to the tumor cells in the presence of the MM serum at all antibody concentrations (Fig. 1D). Using rBCMA, we observed the same concentration dependent inhibitory effects on anti-BCMA antibody binding to MM cells while the same effect was not observed using a non-specific recombinant protein (Fig. 1E). Serum from MM patients with sBCMA levels of 300 ng/mL and 1,000 ng/mL blocked anti-BCMA antibody (5,000 ng/mL [34 nM]) binding to CD138-expressing primary MM tumor cells in a concentration dependent fashion as determined using immunofluorescent staining (Fig. 2).

4. Discussion

BCMA is being increasingly targeted for treatment of plasma cell malignancies [4,14,16–18]. Specifically, chimeric antigen receptor-T-

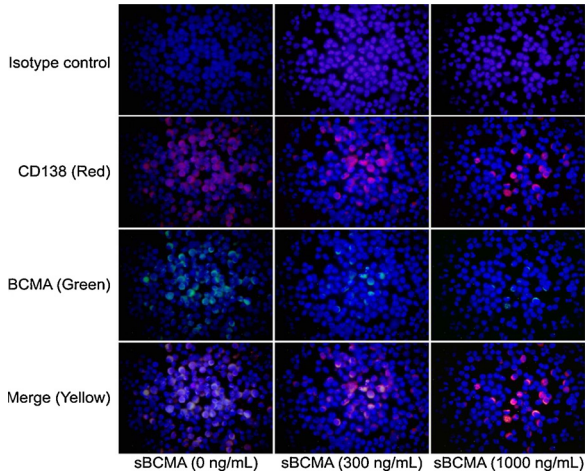

Fig. 2. Immunofluorescent staining of MM BMMCs. Immunofluorescent double staining with anti-BCMA antibody conjugated with FITC at 5,000 ng/mL and CD138 antibody labeled with PE demonstrated that remaining BCMA on MM BMMC membrane from patient #3037 after pre-incubation with MM serum containing increasing concentrations of circulating BCMA (0, 300 and 1,000 ng/mL).

cells, bispecific Tcell engager constructs, and monoclonal antibodies alone or conjugated to toxic chemicals have been used to target BCMA on the surface of tumor cells [14,16–18] with promising clinical activity for RRMM patients [4,13]. In the present study, we have shown that the presence of sBCMA (> 156 ng/ mL) in the blood of RRMM patients [10] may interfere with the efficacy of such therapies. Even though, Carpenter et al. recently had reported that sBCMA did not interfere with anti-BCMA CAR function's anti-MM effects [13], the limitation of the study was the study population. They only tested serum from patients with low concentrations of BCMA (≤ 150 ng/ mL) which is not representative of levels found in the majority of RRMM patients. In fact, we have previously reported that higher sBCMA concentrations are found in most RRMM patients [10]. Consistent with our initial report, we show that in this study the median levels of sBCMA in RRMM patients is 176.0 ng/mL, ranging from 1.8 to 4,865.0 ng/mL. We also demonstrate that sBCMA levels that are present in the majority of RRMM patients interfere with binding of anti-BCMA antibody to MM tumor cells. Specifically, there was a consistent concentration dependent reduction in antibody binding among all patients' MM cells tested at sBCMA levels at or above 156 ng/mL ([26 nM]; Fig. 1B) which is similar to the molar concentration of antibody to which it was mixed (5 μg/mL [34 nM]); the concentration of antibody is present in the blood of patients receiving anti-BCMA antibody treatment [16]. rBCMA also produced a similar concentration dependent reduction in antibody binding to MM tumor cells (Fig. 1E).

The interference of anti-BCMA antibody binding to MM tumor cells from cBCMA could reduce the efficacy of BCMA-targeted approaches. Our findings suggest that it may be important to determine sBCMA levels before using anti-BCMA targeted approaches for MM patients. The initial anti-BCMA antibody dose may require adjustment based on cBCMA levels and may require altering as levels change during treatment. In addition, issues of toxicity may be underestimated if the BCMA-targeted treatment is sequestered by this circulating TNF receptor so that free drug levels may be reduced. Modulation of circulating and tumor bound BCMA levels using γ-secretase inhibitors may improve the efficacy of these approaches especially among patients with levels of cBCMA above the threshold at which interference with antiBCMA antibody binding to MM cells occurs. We have recently shown that γsecretase inhibitors markedly reduce shedding of BCMA from MM cells [12], which may overcome the interference from cBCMA with the efficacy of anti-BCMA-targeted therapies. With the expanding use of treatments targeting BCMA, the clinical value of determining sBCMA levels and possibly decreasing them is likely to become of increasing importance in order to optimize the clinical efficacy of these therapeutic approaches.

Authorship

Contribution: HC and JRB designed the study, analyzed the data and wrote the manuscript. TMS, NX, and NN MG analyzed the data and wrote the manuscript. ML, ES, CM, SP, and GYT performed assays. KU and SB analyzed the data. JC, TH, MZ, EYW, EJT, BZ, RS, MAY, and CSW prepared experimental samples.

Conflict-of-interest disclosure

JRB, HC, ES, ML, and CSW have equity interests in OncoTracker.

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