Inhibitory monoclonal antibody targeting ADAM17 expressed on cancer cells

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Abstract

ADAM17 is upregulated in many cancers and in turn activates signaling pathways, including EGFR/ErbB, as well as those underlying resistance to targeted anti-EGFR therapies. Due to its central role in oncogenic pathways and drug resistance mechanisms, specific and efficacious monoclonal antibodies against ADAM17 could be useful for a broad patient population with solid tumors. Hence, we describe here an inhibitory anti-ADAM17 monoclonal antibody, named D8P1C1, that preferentially recognizes ADAM17 on cancer cells. D8P1C1 inhibits the catalytic activity of ADAM17 in a fluorescence-based peptide cleavage assay, as well as the proliferation of a range of cancer cell lines, including breast, ovarian, glioma, colon and the lung adenocarcinoma. In mouse models of triple-negative breast cancer and ovarian cancer, treatment with the mAb results in 78% and 45% tumor growth inhibition, respectively. Negative staining electron microscopy analysis of the ADAM17 ectodomain in complex with D8P1C1 reveals that the mAb binds the ADAM17 protease domain, consistent with its ability to inhibit the ADAM17 catalytic activity. Collectively, our results demonstrate the therapeutic potential of the D8P1C1 mAb to treat solid tumors.

Introduction

ADAM proteases induce shedding of a variety of membrane-anchored proteins implicated in both health and diseases, notably cytokines, growth factors, chemokines as well as regulators of neurological processes, inflammation and cancer [1–3]. ADAM proteases consist of an N-terminal pro-sequence followed by metalloprotease (M), disintegrin (D), cysteine-rich (C), transmembrane and cytoplasmic domains [4]. The substrate specificity is not imparted by a typical substrate cleavage signature but relies on noncatalytic interactions between the substrate and the ADAM D and C domains [5, 6]. ADAM17, also known as TNF-alpha converting enzyme (TACE), is activated in many cancers, including breast, ovarian, colon and prostate, and in turn triggers oncogenic pathways, including EGFR/ErbB and those underlying resistance to targeted anti-EGFR therapies [1]. Signaling by the EGFR/erbB/HER family is important in regulating proliferation, survival, differentiation and motility during normal development, while dysregulation can promote oncogenesis [7]. The proteolytic release of cell-surface tethered erbB ligands by ADAM17 is a key regulatory switch to trigger EGFR/erbB signaling, which initiates the downstream autocrine signaling that drives tumor progression [8]. Indeed, the erbBs display de-regulated signaling in many human cancers due to over-expression and/or mutations, including in some of the most intractable and common cancers, with EGFR (and HER2) prominent in ovarian, breast, lung and colon cancer, amongst others. In addition, ADAM17-mediated cleavage of erbB2/erbB4 in tumors is associated with constitutive receptor activity and poor prognosis and undermines receptor-targeted therapies [7, 8]. It is anticipated that due to the central role of ADAM17 in oncogenic pathways and drug resistance mechanisms, specific and efficacious monoclonal antibodies against ADAM17...
can be useful for a broad patient population with solid tumors [9–11]. Towards this goal, we generated and characterized an inhibitory anti-ADAM17 monoclonal antibody (mAb) that preferentially recognizes ADAM17 on cancer cells and inhibits solid tumor growth in cell-based and animal-based models.

Materials and methods

Cell lines

All the cell lines described here, except the colon cancer line LIM1215, were purchased from American Type Culture Collection (ATCC). The triple-negative breast cancer (TNBC) cell line MDA-MB-231 was cultured in Dulbecco’s Modified Eagle Medium (DMEM), 10% Fetal Bovine Serum (FBS), 1% Penicillin/Streptomycin (P/S) and 2 mM l-Glutamine. The HER2-overexpressing breast cancer cell line SKBR-3 was grown in McCoy’s 5a, 10% FBS and 1% P/S. The ovarian high-grade serous carcinoma cell line OVCAR-3 was cultured in RPMI-1640, 10% FBS, 1% P/S, 10 mM HEPEs and 0.2 units/ml Insulin while CaOv-3 and SKOV-3 were grown in DMEM, 10% FBS and 1% P/S. The lung adenocarcinoma cell line HCC-827 was cultured in RPMI-1640, 10% FBS and 1% P/S. The glioma cell line U-87 MG was grown in Eagle’s Minimum Essential Medium, 10% FBS and 1% P/S. The LIM1215 colon cancer cell line, derived from a patient with inherited nonpolyposis colorectal cancer [12], was purchased from Cell Bank Australia and cultured in RPMI1640 with 2 mM l-Glutamine, 25 mM HEPEs, 10% FBS, 0.6 µg/ml Insulin, 1 µg/ml Hydrocortisone and 10 µM Thiolglycerol. The cell lines were subcultured according to instruction manuals and tested for mycoplasma contamination before use.

Expression and purification of the human ADAM17 extracellular domain (ECD)

The human ADAM17 cDNA was a gift from Dr. Carl Blobel, Hospital for Special Surgery, New York. The construct comprising of 20–655 amino acids was generated by PCR amplification and cloned into a custom-made pMA152a baculovirus vector [13]. pMA152a is based on the pAcGP67B vector (BD Biosciences) with an incorporated removable Fc-tag (human). Secreted recombinant protein was produced by baculovirus-infected Hi5 insect cell following the protocol provided by BD Biosciences. The C-terminal Fc-tag was used to facilitate protein-A affinity chromatography and removed by thrombin cleavage afterwards. SD-200 size-exclusion chromatography (GE Biosciences) was used to obtain the final purified recombinant protein.

Generation and affinity maturation of monoclonal antibodies binding to the ADAM17 ECD

An ADAM17 ECD construct comprising residues 215–655 was used as an antigen to pan a large naïve human Fab library [14]. Briefly, a naïve human Fab phage display library, constructed from peripheral blood B cells of healthy donors, was used for selection of Fab binders against purified, soluble, ADAM17 ECD conjugated to magnetic beads (Dynabeads M-270 epoxy; DYNAL Inc., New Hyde Park, N.Y.). Amplified libraries of 10^{12} phage displayed Fab were incubated with varying concentrations of ADAM17 ECD for four rounds of panning. Between the rounds, the beads were washed thoroughly to remove non-specifically bound phage. The bead-bound phage was mixed with TGI cells for 1 hour at 37 °C, and the phage was amplified from the infected cells. Randomly picked clones from the infected TGI cells after four rounds of panning were grown in 2YT medium containing 100 µg/ml carbenicillin, 0.2% glucose in 96-well plates by using the automated BioRobotics BioPick colony picking system (Genomic Solutions, Ann Arbor, MI). After the bacterial cultures reached an optical density of 0.5 (at 600 nm), helper phage M13K07 at a multiplicity of infection (MOI) of 10 and kanamycin at 50 µg/ml (final concentration), were added to the medium, and the plates were further incubated at 30 °C overnight in a shaker at 250 rpm. The phage supernatants were then used to identify clones with high binding affinities towards ADAM17 ECD in ELISA-based assays. Two clones, D5 and D8, that bound to ADAM17 ECD with a signal to noise ratio of at least 1 (at A_{450} nm), were selected for expression and purification. The VH and VL of the selected clones were sequenced and plasmids were extracted from these clones for transformation of HB2151 cells. A single colony was picked from the plate containing freshly transformed cells, inoculated into 200 ml 2YT medium containing 100 µg/ml ampicillin and 0.2% glucose, and incubated at 37 °C with shaking at 250 rpm. When the culture OD reached 0.90 at 600 nm, isopropyl-α-thiogalactopyranoside at a 0.5 mM final concentration was added and the culture was further incubated overnight at 30 °C. The bacterial pellet was collected after centrifugation at 8000 × g for 20 min and resuspended in phosphate-buffered saline pH 7.4, (PBS) containing 0.5 mM polyoxymin B (Sigma-Aldrich, St. Louis, MO). After 30 min incubation the bacterial slurry was centrifuged at 25,000 × g for 25 min at 4 °C, and the supernatant was used for Fab purification with a protein G column (Sigma-Aldrich, St. Louis, MO). For conversion of D5 and D8 Fabs to IgG1 format, the heavy and light chains were amplified and recloned in the PDR12 vector for whole immunoglobulin-G1 (IgG1) expression. The construct was then transfected into the Freestyle 293 expression system. Whole IgG1 was purified from the culture supernatant using protein G matrix.

The affinity maturation of the D8 and D5 antibodies was carried out by Lake Pharma, Inc. using targeted mutagenesis. After 3 rounds of selection, the affinity matured clones were highly enriched, and the affinity improvement was confirmed using parental D5 and D8 mAbs as references. The matured clones had 10-fold or better improvement in binding affinity over the parental clones and bound to human ADAM17 with Kds of 50–80 pM (Lake Pharma, Inc.). The EC50 values and sequencing results were used to select a panel of five affinity matured clones for further studies (See Supplementary Table S1). The MED13622 (MedImmune/AstraZeneca) anti-ADAM17 mAb was provided by the Tri-Institutional Therapeutics Discovery Institute (Memorial Sloan Kettering, The Rockefeller University and Weill Cornell Medical College, New York).

Fluorescent peptide cleavage assay

The purified ADAM17(ECD)-binding mAbs D8P1C1 and D5P2A11, as well as the mAb MED13622, were buffer-exchanged into 25 mM Tris, pH 9.0, containing 2 µM ZnCl2, and 0.005% (w/v) Brij-35. ADAM17-antibody complexes were formed at a 1:1 molar ratio prior to the assay. The assay was carried out by mixing 50 µM of a fluorogenic peptide substrate Mca-PLAQAV-Dpa (R&D Systems Cat# E5003) with ADAM17-antibody complexes at 37 °C and monitoring the progress of the enzymatic reaction by fluorescence emission (excitation 320 nm and emission 405 nm) over a time course of 1 h using a SpectraMax M5. ADAM17 (ECD) alone was used as a positive control. The substrate peptide was derived from TNFαp1a and contains a highly fluorescent 7-methoxyxycarbomarin group and a quencher group, 2,4-dinitrophenyl. ADAM17 cleaves the amide bond between the fluorescent and the quencher group causing an increase in fluorescence (R&D Systems, Cat Number E5003). [15, 16].

Negative staining em analysis

Protein complex of ADAM17 ectodomain with D8P1C1 Fab was prepared by mixing the two proteins at a 1:1 molar ratio, then diluted with a buffer containing 10 mM HEPEs, pH 7.4, 150 mM NaCl, adsorbed to a freshly glow-discharged carbon-coated copper grid, washed three times with the same buffer, and stained three times with 0.75% uranyl formate. Images were collected at a magnification of 57,000 using EPU on a Thermo Fisher Talos F200C microscope equipped with a 4k x 4k CETA 16 M camera and operated at 200 kV. The pixel size was 2.5 Å for
the CETA camera. Image processing, particles picking, reference-free 2D classification, 3D reconstruction and refinement were performed using cryoSPARC. The structure was visualized using Chimera.

Alamar Blue cell viability assay

Cells (breast/ovarian/colon/glioma/lung-adenocarcinoma) were harvested in the log phase of growth (after 3 days of culturing). The cell count was determined and was adjusted to 5 × 10^5 cells/ml. The cells were allowed to adhere and grow for 24 h in 96-well cell culture plates, treated with test agents, in this case, the affinity matured ADAM17 mAbs D8P1C1, D5P2A11 and their parental clones D8 and D5. The cells were allowed to grow for additional 38 h. Cells not treated with mAbs were used as a control. Alamar Blue (Bio-RAD Laboratories) (10% of the well volume) was added aseptically. Cultures containing Alamar Blue were incubated for 6 hrs and cell proliferation was measured spectrophotometrically by absorbance at 570 and 600 nm. Cell viability was calculated using the following formula:

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\text{Percentage difference between treated and control cells} = \frac{(O_2 \times A_1) - (O_1 \times A_2)}{(O_2 \times P_1) - (O_1 \times P_2)} \times 100
\]

\( O_1 \) = molar extinction coefficient (E) of oxidized alamarBlue® (Blue) at 570 nm, \( O_2 \) = E of oxidized alamarBlue® at 600 nm, \( A_1 \) = absorbance of test wells at 570 nm, \( A_2 \) = absorbance of test wells at 600 nm, \( P_1 \) = absorbance of positive growth control well (cells plus alamarBlue® but no test agent) at 570 nm, \( P_2 \) = absorbance of positive growth control well (cells plus alamarBlue® but no test agent) at 600 nm [17].

Cell-based ELISA assays to gage the binding of anti-ADAM17 mAb D8P1C1 to cancer cell lines

Cellular ELISA [18] was performed to gage the binding of the anti-ADAM17 mAb D8P1C1, relative to the binding of the MEDI3622 mAb, to ADAM17 expressed on the cell surface of cancer cell lines (breast, ovarian, colon, glioma, adenocarcinoma), as well as to HEK293 cells and HEK293 cells transfected with full-length human ADAM17. Briefly, 5 × 10^4 cells/well were immobilized on 96-well ELISA plates (Greiner bio-one) with 1% paraformaldehyde for 2 hrs at 37 °C. The plate was washed thrice with PBS and blocked for 2 hrs at room temperature with 4% non-fat dry milk. The anti-ADAM17 mAbs were added in varying concentrations. Mouse mAb conjugated to HRP and recognizing human IgG was used as a secondary antibody (Abcam) and color was developed using the TMB substrate kit (Thermo Scientific). The data was recorded at 450 nm.

In vivo antitumor efficacy studies

All cells were grown in monolayer culture, harvested by trypsinization, and implanted subcutaneously into the right flank of 6- to 8-week-old female athymic nude mice for MDA-MB-231, and NSG mice for SKOV-3 (n = 5). Approximately 10 million cells (MDA-MB-231 or SKOV-3) were injected per mouse. Mice were randomized into 5 mice per group. When tumors reached 100 to 150 mm³, the anti-ADAM17 mAb D8P1C1, prepared by diluting with sterile PBS, was injected intraperitoneally, twice a week for four weeks, according to body weight. Sterile PBS was used as a control. Tumor volume was determined by external caliper and calculated by the modified ellipsoidal formula: \( V = \frac{1}{2} \times (\text{Length} \times \text{Width}^2) \). Antitumor efficacy was calculated as (1-\( DT/DC \)) X100, where \( DT \) is the final tumor volume minus the starting tumor volume from the treatment group and \( DC \) is the final tumor volume minus the starting volume of the control group [19]. Error bars were calculated as SEM. The mouse body weight and general health were monitored daily, and the experiments were carried out in accordance with Association for Assessment and Accreditation of Laboratory Animal Care and MSKCC Institutional Animal Care and Use Committee guidelines.

Statistical analysis

The data from all in vitro and cell-based assays described, including Alamar blue, fluorescent peptide cleavage and ELISA-based assays, are representative of triplicate determinations and two independent experiments. Unpaired two-tailed Student’s t-test was performed to determine the significance of the results.

Results

The recombinant ADAM17 ectodomain is monomeric in solution

The ectodomain (ECD) of ADAM17 (residues 20–655) was cloned in a custom-made vector, pMA152a [13] for baculovirus expression. The PCR amplified product was fused to the envelope surface glycoprotein gp67 signal sequence and the ADAM17(ECD) was purified from the culture supernatant using protein A Sepharose and SD-200 (size-exclusion chromatography). ADAM17(ECD) elutes as a monomer with a native molecular weight of 65 kDa. The prodomain is cleaved during secretion of the protein into the culture medium. The purified ADAM17 (ECD) recombinant protein was N-terminally sequenced confirming that the final product (amino acid residues 215–655) consists of the metalloprotease (MP), disintegrin (D) and cysteine rich (C) domains, Fig. 1A. The matured (lacking the pro domain) ECD was used as an antigen to raise a panel of ADAM17 specific monoclonal antibodies.

Generation of anti-ADAM17 monoclonal antibodies

Construction of a large naïve phage-displayed Fab library was achieved through one-step cloning: SFI was used as the cloning enzyme, since it can keep almost all the antibody gene repertoire intact during the cloning procedure. To fully cover each of the human antibody genes, carefully selected primers, matching the conserved N- and C-terminal region of the heavy- and light-chains, were used for the amplification of each of the gene families separately [14]. Two Fab’s, D8 and D5, were selected based on their binding specificities and affinities towards the ADAM17 ECD (using ELISA). Cross-reactivity with closely related ADAM’s, including ADAM10 and ADAM19 were also evaluated by ELISA-based screening. The selected Fab specifically recognize the human and mouse ADAM17 ectodomain and did not bind to ADAM10 or ADAM19 (data not shown). They were then reformatted to a human IgG1 framework. Affinity maturation of D8 and D5 were carried out by Lake Pharma using targeted mutagenesis. A panel of 5 affinity matured clones for D8 and D5 were selected based on sequencing results (selecting distinct clones), EC50 and Kd values.

Two affinity matured anti-ADAM17 antibodies, D8P1C1 and D5P2A11, efficiently inhibit the proliferation of a triple-negative breast cancer cell line

ADAM17-dependent EGFR ligand (TGF-alpha) shedding is an important proliferative signal in breast cancer and ADAM17 is known to play a key role in the progression of triple-negative breast cancer (TNBC) [20]. Indeed, it has been shown that small molecule inhibitors to ADAM17 block invasion, migration and colony formation of several triple-negative breast cancer cell lines [21]. We, therefore, evaluated the anti-tumor potential of the five affinity-matured anti-ADAM17 mAbs (see above) in an Alamar blue cell-based assay using the TNBC cell line MDA-MB-231 that is HER2 negative and EGFR positive [22]. The results reveal that D8P1C1 and D5P2A11 are the most effective clones in inhibiting the proliferation of the TNBC cell line with IC50 values of 0.037 and 0.069 µg/ml, respectively (Fig. 1B, C, Supplementary Table S1). Based on these experiments, we selected D8P1C1 and D5P2A11 for further studies. The IC50 values of the five affinity matured
clones and their parental counterparts, D8 and D5, are shown in Table S1. Noteworthy, the IC50 values of D8P1C1 and D5P2A11 are 5 to 10-fold lower than the parental D8 and D5 clones.

D8P1C1 binds to the catalytic domain of ADAM17 and inhibits its proteolytic activity

In a fluorescent peptide cleavage assay, the mAb D8P1C1 significantly inhibits the catalytic activity of ADAM17, Fig. 2A. At the concentrations used, D8P1C1 is twice as effective as the MedImmune/AstraZeneca mAb MED13622 in inhibiting substrate cleavage. We next carried out negative-stain EM analysis with ADAM17(ECD) bound to a Fab fragment of D8P1C1. The D8P1C1 Fab fragment was generated by restricted papain cleavage of the whole IgG. The ADAM17(ECD)/Fab complex was prepared by mixing the two components in 1:1 molar ratio. Both 2D class averaging and 3D reconstruction show detailed features corresponding to the catalytic, disintegrin and cysteine rich ADAM domains, as well as the bound Fab, Fig. 2B. In the calculated 3D EM density map, we docked the X-ray crystal structures of the ADAM10 disintegrin and cysteine rich domains (PDB ID 5L0Q) [23], as well as that of the ADAM17 catalytic domain (PDB ID 3EWJ) [24]. These results show that the Fab D8P1C1 binds to the catalytic domain of ADAM17 (Fig. 2B and Supplementary Fig. S1). Further studies are underway to obtain atomic-resolution structure of the complex.

D8P1C1 and D5P2A11 inhibit the proliferation of multiple EGFR-overexpressing cancer cell lines

We next performed cell viability assays with other cancer cell lines, including OVCAR-3, SKOV-3, CAOV-3 (ovarian) and SKBR-3 (breast), U87-MG (glioma), LIM1215 (colon) and HCC-827 (non-small cell lung cancer or NSCLC), Fig. 3A, B. These cell lines are known to overexpress EGFR/HER2 [22, 25]. The ovarian cancer cell lines OVCAR-3 and CAOV-3 represent high-grade serous carcinoma (HGSC) type, while SKOV-3 belongs to the non-HGSC type [25]. Though high-grade serous carcinoma is the most prevalent amongst ovarian cancer patients, the...
non-serous types are known to migrate and invade more aggressively [25]. The Alamar blue assays show that the two anti-ADAM17 mAbs (D8P1C1 and D5P2A11) cause varying degree of proliferation inhibition, Fig. 3 A, B. Specifically, they are more efficient in inhibiting the HER2-dependent breast cancer cell line SKBR-3 and the EGFR-expressing lines HCC-827, LIM1215 and U87-MG, as compared to the ovarian cancer cell lines. For instance, at a concentration of 20 µg/ml, D8P1C1 caused 70% and 63% proliferation inhibitions of SKBR-3 and HCC-827, respectively, compared to 35% inhibition of OVCAR-3. D8P1C1 specifically recognizes ADAM17 on cancer cells

The ADAM activity is proposed to be regulated via transitions between two distinct conformations, “active” or “open” and “latent” or “closed” [26] supported by “open” and “closed” conformations observed in the crystal structures of related snake venom metalloproteinases [27]. These two radically different conformations are proposed to result from alternative disulfide-bonding arrangements of the D-C region [27, 28].

Previously, our studies with ADAM10 and the ADAM10-specific mAb 8C7 highlighted how 8C7 recognizes the active conformation of ADAM10 prevalent in tumors, as opposed to the inactive/latent ADAM10 conformation present in normal tissues [23]. To assess whether D8P1C1 also preferentially recognizes the tumor-specific ADAM17 conformation, we measured its binding to several human tumor cell lines relative to the binding of a commercial mAb (MED13622, MedImmune/AstraZeneca) that is not conformation specific. The cell-based ELISA [18] results with TNBC, ovarian, glioma, colon and non-small cell cancer cell lines document that D8P1C1 indeed preferentially binds to ADAM17 expressed on human tumor-derived cells, Fig. 4, as compared to ADAM17 expressed on HEK293 cells.

D8P1C1 caused tumor growth inhibition of the TNBC cell line MDA-MB-231 and the ovarian cancer cell line SKOV-3 in mouse xenograft studies

We evaluated the anti-tumor potential of D8P1C1 in vivo mouse models of TNBC using MDA-MB-231 cells. Administration of D8P1C1 at a dose of 15 mg/kg results in 78% percent tumor growth inhibition (Fig. 5). There were no toxicity effects in animals, gauged from no loss in mouse weight or presence of visible diarrhea, Fig. 5B, despite D8P1C1 binding equally well to human and mouse ADAM17 in ELISA-based assays (data not shown). This confirms that, although ADAM17 is present on a variety of cells, the inhibitory D8P1C1 mAb selectively targets tumors, without significant side effects or toxicity.

Finally, in an ovarian cancer xenograft model with SKOV-3 cells, D8P1C1 causes 45% tumor growth inhibition at a higher dose of 60 mg/kg, which we selected to assess toxicity at high concentrations. As above, no discernible toxicity effects were observed (Fig. 6).

Discussion

ADAM17 is a member of the ADAM family of metalloproteinases. It cleaves over 80 substrates that are involved in tumor progression, inflammation and neurological diseases. In addition to erbB ligands as discussed above, substrates include Jagged1 [29], Glypican [30] and the C-MET receptor [31] that are primarily responsible for metastasis, cell proliferation and maintenance of stem cell populations in a wide variety of solid and hematological cancers [21]. ADAM17 also cleaves substrates, such as Neogenin [32], Syndecan-4 [33] and Glycoprotein VI, which augment inflammatory pathways [34]. It has been documented that ADAM17 sheds the Fc receptor CD16 that is present on peripheral blood natural killer (NK) cells and over-activation of ADAM17 dampens the effector functions of NK cells by downregulating the cell surface expression of CD16. ADAM17 antagonists play a major role in restoring...
the effector functions of NK cells [35]. Furthermore, ADAM17 is upregulated or mutated in many cancers (Fig. S2). Consequently, ADAM17 is considered to be an attractive target for therapeutic intervention. Several small molecule inhibitors targeting the catalytic domain of ADAM17 have been previously developed, such as Apratastat (Wyeth pharmaceuticals), DPC 333 (Bristol-Myers Squibb Company) and INCB7839 (Incyte corporation), but they failed in clinical trials due to their toxicity and lack of specificity [24]. This is mainly because the inhibitors also target a close homolog of ADAM17, ADAM10, and some essential matrix metalloproteinases, such as MMP2, MM12, MMP15 [24]. Further chemical modifications of these compounds are in progress. A quinoline-derivative of the hydroxamate-based inhibitors DPC-33 and Apratastat was shown to inhibit proliferation of human keratinocytes with an IC50 of 3 nM and has been selected as a clinical candidate for psoriasis. Non-hydroxamate and non-zinc binding inhibitors of ADAM17 have also been investigated in cell-based assays but none has been tested in clinical trials [36]. Thus, monoclonal antibodies targeting ADAM17 are suggested to provide a better arsenal to deter cancer progression. To date, two anti-ADAM17 inhibitory mAbs, D1(A12) and MED13622, have shown moderate to high efficacy in vitro and in vivo tumor models of triple-negative breast, head and neck squamous cell epithelial, as well as ovarian and esophageal, cancers [37–39]. Binding and modeling studies indicate that D1(A12) binds to both the catalytic and noncatalytic ADAM17 domains while MED13622 binds a unique hairpin loop in the ADAM17 metalloprotease domain. This loop is absent in other ADAMs, ADAMTSs and MMPs explaining the basis for the mAb’s specificity [40].

Here we describe the generation and anti-tumor potential of a new...
anti-ADAM17 mAb, D8P1C1. D8P1C1 inhibits proliferation of a host of cancer cell lines, including TNBC and HER2-overexpressing breast, ovarian, glioma, colon and non-small cell lung cancer lines. Though the inhibitory effect is modest in ovarian, glioma and colon cancer cells, it is significant in the case of the two breast cancer cell lines MDA-MB-231 (TNBC) and SKBR-3 (HER2 overexpressing), as well as the non-small cell lung cancer cell line HCC-827 that has an acquired mutation in the tyrosine kinase domain (E746-E750) [41]. Importantly, in an in vivo xenograft model of TNBC, D8P1C1 causes 78% tumor growth inhibition. On the other hand, in an in vivo xenograft model of SKOV3 (ovarian cancer) the mAb exhibits moderate inhibition. This could be attributed to the prevalence of the MUC-16 signaling pathway, which promotes progression of ovarian cancers independently of the EGFR-ADAM17 signaling events [41]. MUC-16 binds to adhesion molecules, including mesothelins and glectins, and activates the AKT/ERK pathways contributing to the adverse outcomes in ovarian cancer [42].

Unlike the previously described anti-ADAM17 mAbs, D8P1C1 specifically recognizes ADAM17 expressed on cancer cell lines. This suggests that the mAb D8P1C1 selectively binds to an activated conformation of ADAM17 prevalent on cancer cells [23, 26]. An in vitro fluorescent peptide cleavage assay reveals that D8P1C1 acts via inhibiting the ADAM17 catalytic activity, and a negative-stain EM structure of the D8P1C1/ADAM17 complex, derived from 3D-reconstruction, shows that D8P1C1 binds to the ADAM17 protease domain. The catalytic inhibition studies also document that D8P1C1 is more efficient in blocking peptide cleavage by ADAM17 than the MedImmune/AsstraZeneca mAb MED13622 mAb. It is known that TNBCs are not sensitive to targeted therapeutics used for HER2-positive and ER+positive tumors [43]. Likewise, in the case of non-small cell lung cancer, the currently used anti-EGFR therapeutic monoclonal antibodies, including Cetuximab and Panitumumab, although effective in killing the cancer cells, exhibit severe side effects, including rash and diarrhea. The observation that D8P1C1 is tumor-conformation-specific and exhibits no discernible toxicity effect in animal models, validates future efforts to develop D8P1C1-based reagents for treatment of TNBC, NSCLC and solid tumors at large.

Declaration of Competing Interest

The authors declare that they have no competing interest.

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Author contribution

Nayanendu Saha: conceptualization, writing original draft, investigation. Kai Xu, Zhongyu Zhu, Yan Xu: data curation, analysis, investigation, editing, methodology. Dorothea Robev and Teja Kalidindi: data curation. Elisa de Stanchina, Naga Vara Kishore Pillarsetty, Juha Himanen and Dimiter S Dimitrov: formal analysis of data, review and editing. Dimitar B. Nikolov: supervision, formal analysis, review and writing, funding acquisition.

Supplementary materials

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