Antibody targeting of ADAM8 for treatment of triple-negative breast cancer

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Abstract

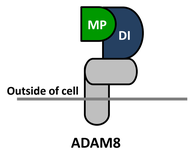
Triple-Negative Breast Cancer (TNBC) accounts for 25% of breast cancer deaths. Current therapeutic options are restricted to chemotherapy (CT), surgery and radiation, which fail to block disease progression. Recently, we identified the cell surface protein ADAM8 as a critical driver of TNBC tumor growth and metastasis (EMBO Mol. Med. 6:278, 2014). Elevated *ADAM8* levels correlate with poor breast cancer patient outcome. One third of TNBC patient samples have high ADAM8 levels, while it is undetectable in normal breast tissue. Half of all breast cancer metastases express ADAM8. Mechanistically, the ADAM8 Metalloproteinase (MP) and Disintegrin (DI) domains promote angiogenesis needed for tumor growth, and cancer cell adhesion to and invasion through the endothelium needed for dissemination, respectively. Using a prototype reagent in mice, we validated a unique antibody-based strategy that simultaneously targets ADAM8 MP/DI activities as an effective therapeutic intervention in TNBC.

We have now prepared a panel of pre-clinical, highly specific anti-human ADAM8 mouse monoclonal antibodies (termed ADPs) of either IgG1 or IgG2 subclass with dual MP/DI antagonist activity. Here the characterization of these antibodies is described.

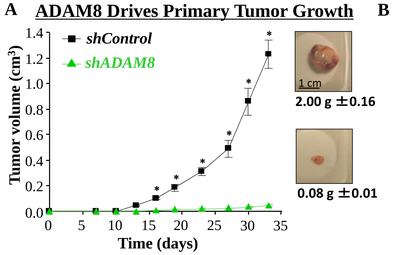
ADAM8 is a novel target for TNBC treatment

ADAM8

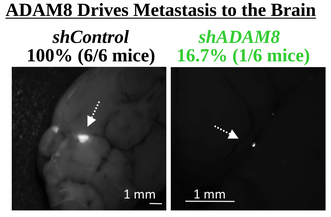
- Membrane-anchored protein synthesized as a proform that autocatalytically clips off its prodomain resulting in an active processed form
- Has two functional domains: the MP (Metalloproteinase) and the DI (Disintegrin) domain
- Involved in growth factor shedding, cell adhesion, cell migration, and proteolysis of the extracellular matrix
- Non-essential under physiological conditions as evidenced by the lack of phenotype of ADAM8 deficient mice
- Plays a key role in TNBC tumor growth and metastasis



A **ADAM8 Drives Primary Tumor Growth**



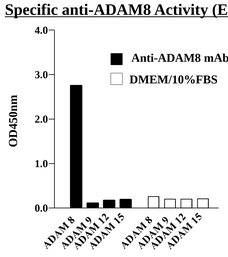
B **ADAM8 Drives Metastasis to the Brain**



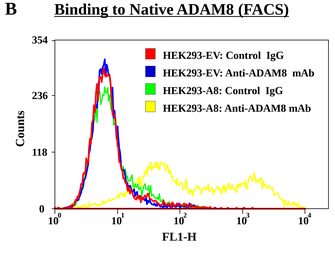
A) Growth of tumors derived from MDA-MB-231 TNBC cells with ADAM8 knockdown. MDA-MB-231 derived *shControl* and *shADAM8* cells were injected into the mammary fat pad (MFP) of female NOD/SCID mice (n = 7/group). Tumor volume was measured 2x/week (mean ± s.e.m.). *P = 1.4E^{-6}, Student's t-test. At the end of the experiment, tumors were photographed and weighed (mean ± s.e.m.). **B) Brain metastases.** Presence of brain metastases was examined by fluorescent microscopy (n = 6/group). Representative photographs are shown.

Novel mouse monoclonal antibodies have anti-ADAM8 specific activity

A **Specific anti-ADAM8 Activity (ELISA)**



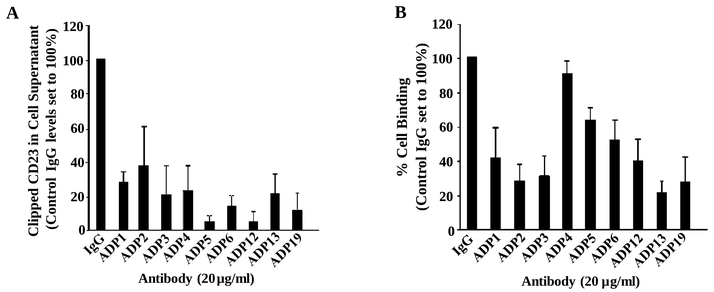
B **Binding to Native ADAM8 (FACS)**



Similar activity was seen with the full panel of 17 novel anti-ADAM8 mAbs

A) ELISA assay demonstrating specific binding of novel anti-ADAM8 antibody. ELISA plates, coated with 0.5 µg/ml of the indicated recombinant ADAM proteins, were incubated with one of 17 novel anti-ADAM8 mouse monoclonal antibodies (mAbs), termed ADPs. Binding was detected using an anti-mouse-HRP secondary antibody. **B) FACS histogram of antibody binding to native ADAM8 expressed on the surface of HEK293 cells.** HEK293-empty vector (EV) or HEK293-ADAM8 (A8) expressing cells were incubated with anti-ADAM8 antibody and subjected to FACS analysis.

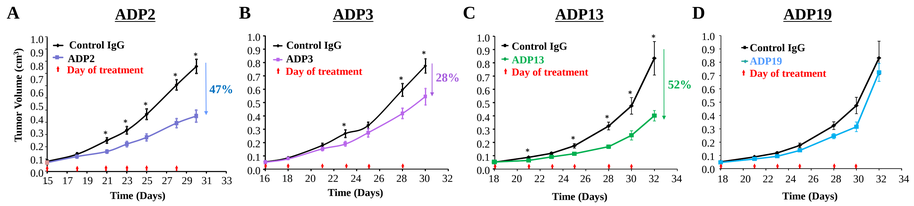
Proprietary novel dual antagonist antibodies inhibit both the MP and DI domains of ADAM8



A) Activity of ADP antibodies against the MP domain of ADAM8. HEK-293 cells were co-transfected to overexpress ADAM8 and the ADAM8-target protein CD23 (HA-tagged). Cells were then treated with 20 µg/ml of the indicated ADP antibodies or IgG control in serum free media for 16 h. Supernatants were collected and subjected to Western blotting to measure the amount of released cleaved CD23, using an anti-HA antibody. The average of three experiments is presented as percent relative to control IgG (IgG) samples set to 100%.
B) Activity of ADP antibodies against the DI domain of ADAM8. Antibody-mediated inhibition of ADAM8 DI activity was tested by assessing the ability of CHO cells expressing α9β1-Integrin to bind to recombinant human ADAM8 coated plates. ELISA plates were coated overnight with 1.5 µg recombinant human ADAM8. Coated plates were treated with 20 µg/ml of IgG control or anti-ADAM8 ADP antibodies, in triplicate, overnight at 4°C. Plates were washed and a single cell solution of 10^5 α9β1-integrin CHO cells in 1% BSA supplemented DMEM media was added and incubated for 1 h at 37ºC. Wells were then washed and photographed at 10X. Number of cells binding to each well was counted manually using the count tool in Adobe Photoshop software. The average of three experiments is presented as percent relative to control IgG samples set to 100%. ADP antibody-mediated inhibition of ADAM8 DI activity was also assessed by the ability of MDA-MB-231 cells to migrate through a layer of HUVEC cells and similar results were obtained.

Based on these findings, 4 antibodies (ADP2, ADP3, ADP13, and ADP19) were selected for single-dose efficacy testing on pre-existing TNBC tumors in mice

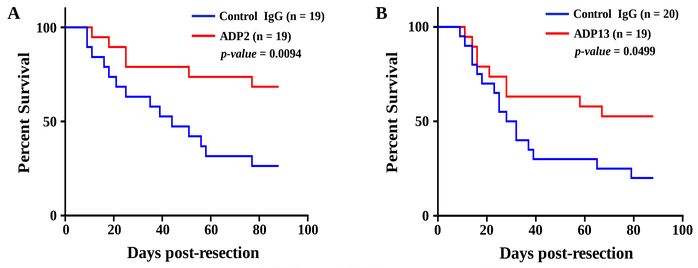
Dual antagonist anti-ADAM8 antibodies cause significant reduction in growth of pre-existing TNBC tumors



Tumor growth in mice treated with ADP antibodies. ADAM8-positive MDA-MB-231 TNBC cells were implanted in the MFP of female NOD/SCID mice (n=7/group). The tumors were allowed to grow until they reached a size of 50-70 mm^3. Animals were then treated with 10 mg/kg of either **(A) ADP 2, (B) ADP3, (C) ADP13, (D) ADP19** or their respective isotype-matched controls in i.p. injection 3x/week. Tumor volume was measured on the indicated days (mean ± s.e.m.). *P < 0.05 Student's t-test. Treatment with ADP2, ADP3, and ADP13 resulted in significant reductions in tumor volume of 47%, 28%, and 52%, respectively. ADP19 did not reduce tumor growth.

Based on these findings, ADP2 and ADP13 were selected as our lead antibodies.

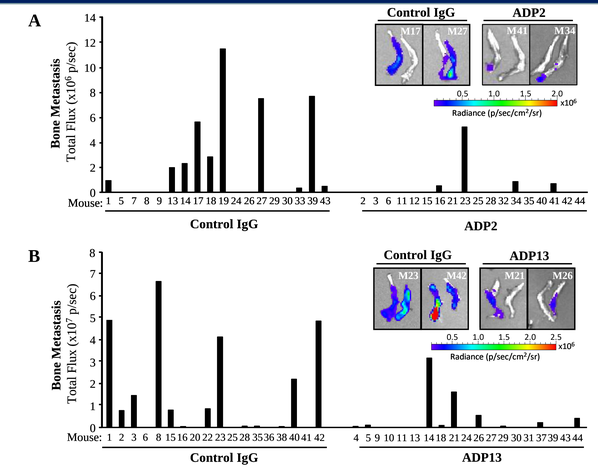
ADP2 and ADP13 anti-ADAM8 dual antagonist antibodies significantly increase recurrence-free survival in TNBC mouse model



Recurrence-free survival in mice treated with ADP2 and ADP13 antibodies vs IgG controls. Luciferase tagged MDA-MB-231 cells were injected into the MFP of NOD/SCID mice. When tumors reached a volume of 50-70 mm^3, mice were randomly distributed into two groups and treated with 10 mg/kg of either **ADP2 (A), ADP13 (B)** or their control IgG 2x in the span of a week. Tumors were resected when they reached a volume of ~200 mm^3. Treatment was continued 3x/week for 12 weeks. Tumor recurrence was measured by palpation and Kaplan-Meier curves generated using Prism 7 software; p-values were calculated using a log-rank test. Overall survival (endpoint defined as recurrent tumor of 900 mm^3) was also significantly increased with ADP2 and ADP13 treatment.

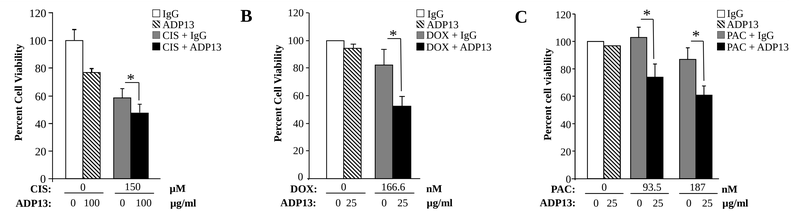
ADP2 and ADP13 significantly increase recurrence-free survival, and overall survival

Anti-ADAM8 dual antagonist antibodies decrease metastasis



Metastasis in mice treated with ADP antibodies. Mice from our MDA-MB-231-luciferace tagged TNBC recurrence model described in the previous panel were sacrificed when recurrent tumors reached a size of 900 mm^3. Tissues were dissected out and scanned for presence of metastases using biophotonic imaging. Total flux was calculated using Living Image (IVIS) software and graphed to demonstrate presence and extent of bone metastases in mice treated with ADP2 **(A)** or ADP13 **(B)** antibody vs control IgG. Representative images for each antibody are given.

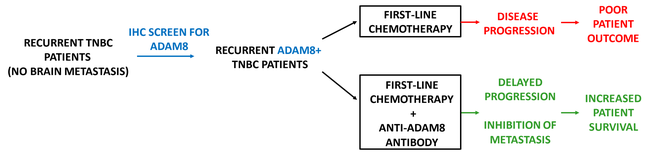
ADAM8 inhibition sensitizes TNBC cells to Cisplatin, Doxorubicin and Paclitaxel



Combination treatment of a monoclonal anti-ADAM8 antibody with chemotherapeutic agents (CT). MDA-MB-231 cells were cultured as spheroids in low attachment 96-well plates for 3 days followed by incubation with 25 µg/ml ADP13 or control IgG for 24 h and then with the indicated doses of Cisplatin (CIS) **(A)**, Doxorubicin (DOX) **(B)** or Paclitaxel (PAC) **(C)**. After 72 h of treatment, cell viability was measured using a 3D MTT assay kit (Trevigen). *P < 0.05 Student's t-test.

The best combination of chemotherapy + anti-ADAM8 antibody will be identified *in vivo*

Strategy for future clinical testing



Combining anti-ADAM8 antibodies with chemotherapy for recurrent TNBC. Our strategy is to focus on recurrent ADAM8-positive TNBC as the first indication. Patients will be selected using a companion diagnostic which we are co-developing. The strategy and expected benefits are illustrated above.

Conclusions

Proprietary anti-ADAM8 dual antagonist antibodies were developed. These ADPs:

- Inhibit both the MP and DI domains of ADAM8
- Cause significant tumor reduction in pre-existing breast tumor models
- Improve recurrence-free survival
- Decrease metastasis
- Sensitize TNBC cells to standard chemotherapeutic agents

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