Inhibition of Cell Adhesion by a Cadherin-11 Antibody Thwarts Bone Metastasis

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Abstract

Cadherin-11 (CDH11) is a member of the cadherin superfamily mainly expressed in osteoblasts but not in epithelial cells. However, prostate cancer cells with a propensity for bone metastasis express high levels of cadherin-11 and reduced levels of E-cadherin. Downregulation of cadherin-11 inhibits interaction of prostate cancer cells with osteoblasts in vitro and homing of prostate cancer cells to bone in an animal model of metastasis. These findings indicate that targeting cadherin-11 may prevent prostate cancer bone metastasis. To explore this possibility, a panel of 21 monoclonal antibodies (mAb) was generated against the extracellular (EC) domain of cadherin-11. Two antibodies, mAbs 2C7 and 1A5, inhibited cadherin-11–mediated cell–cell aggregation in vitro using L-cells transfected with cadherin-11. Both antibodies demonstrated specificity to cadherin-11, and neither antibody recognized E-cadherin or N-cadherin on C4-2B or PC3 cells, respectively. Furthermore, mAb 2C7 inhibited cadherin-11–mediated aggregation between the highly metastatic PC3-mm2 cells and MC3T3-E1 osteoblasts. Mechanistically, a series of deletion mutants revealed a unique motif, aa 343-348, in the cadherin-11 EC3 domain that is recognized by mAb 2C7 and that this motif coordinated cell–cell adhesion. Importantly, administration of mAb 2C7 in a prophylactic setting effectively prevented metastasis of PC3-mm2 cells to bone in an in vivo mouse model. These results show that targeting the extracellular domain of cadherin-11 can limit cellular adhesion and metastatic dissemination of prostate cancer cells.

Implications: Monotherapy using a cadherin-11 antibody is a suitable option for the prevention of bone metastases. Mol Cancer Res; 11(11); 1401–11. ©2013 AACR.

Introduction

Advanced prostate cancer often metastasizes to distant organ sites with bone being the most commonly affected site (1). One of the contributors to the lethal progression of the disease is the abnormal expression of cadherin-11 (Cad11) in prostate cancer cells (2). Cad11 is the physiologic cadherin molecule expressed on osteoblasts (3). However, our previous studies showed that prostate cancer cells, especially those in bone metastases, often switch the cadherin type from E-cadherin to Cad11, due to epithelial–mesenchymal transition (EMT; ref. 2). This EMT transition enables prostate cancer cells to interact with osteoblasts in bone (4). Moreover, downregulation of Cad11 in highly metastatic PC3-mm2 cells with Cad11-specific short hairpin RNA significantly decreased the incidence of PC3-mm2 metastasis to bone in an animal model of metastasis (2). These findings suggest that targeting Cad11-mediated cell–cell interaction may be a promising strategy in preventing prostate cancer bone metastasis.

Inhibition of Cad11-mediated prostate cancer and osteoblast interaction can be achieved through small molecules or antibodies that recognize the extracellular domain of Cad11. Because prostate cancer is often detected early and there is nearly a 10-year “window” during which anti-metastasis therapy would be useful as “secondary prevention”, the stability and efficacy of the targeting agents will be key factors for the feasibility and success of the treatment. As compared with small molecules, antibodies are more stable in the circulation and thus more suitable for chronic administration in a prophylactic setting for the prevention of metastases in patients with a high risk of developing bone metastasis. The objective of this study is to determine the...
feasibility of developing an antibody-based prevention strategy that targets Cad11.

Three tasks need to be fulfilled in the early stages of developing antibodies that target Cad11-mediated cell–cell adhesion. The first task is to develop an antibody that has the desired activities for performing proof-of-concept studies. The second task is to test the concept that targeting the extracellular domain of Cad11 is able to prevent prostate cancer metastasis in an animal model system in vivo. Although our previous studies showed that Cad11 knockdown inhibits prostate cancer metastasis in an animal model of metastasis, it was not clear whether the inhibition of extracellular interactions is sufficient to inhibit metastasis to bone. The third task is to identify the region/motif in the extracellular domain of Cad11 recognized by the antibodies. Identification of this motif will lay the foundation for developing more effective antibodies that target Cad11-mediated cell–cell interaction for clinical application.

In this study, we generated 21 antibodies against the extracellular domain of Cad11 and identified two promising candidates from this panel. We identified a previously unknown adhesion motif in the extracellular domain of Cad11 that is recognized by both antibodies. We further conducted animal studies with one of the characterized antibodies and obtained evidence that targeting this unique motif in the third extracellular domain (EC3) of Cad11 by the antibody is effective in reducing prostate cancer metastasis to bone.

Materials and Methods

Materials

C4-2B4-Cad11 expressing Cad11 and GFP, and PC3-mm2-Luc, expressing luciferase and GFP, were generated as described previously (2, 4). PC3-mm2 and C4-2B4 cell lines were confirmed by fingerprinting. Goat anti-Cad11 polyclonal antibody was purchased from R&D Systems.

Cell aggregation assay

L-cells (CCL1.3) expressing Cad11 (L-Cad11) were generated by infecting L-cells with recombinant retroviruses expressing Cad11 and GFP as previously described (5). Control L-vector cells express only GFP. L-cells or L-Cad11 cells were released from culture plates using Cellstripper (Cellgro, Mediatech, Inc.) for 10 to 12 minutes. Control L-vector cells express only GFP. L-cells or viruses expressing Cad11 and GFP as previously described were conduced animal studies with one of the characterized Cad11 that is recognized by both antibodies. We further

expression and purification of Cad11-Fc, Cad11-his7 recombinant proteins and generation of anti-Cad11 monoclonal antibodies

Cad11-Fc protein, containing the extracellular domain of Cad11 fused to Fc, was prepared as described (5). Cad11-his7 protein, containing the extracellular domain of Cad11 with a 7-histidine tag in the C-terminus, was constructed as follows. The pBMN-(OB-CAD-Fc)-I-GFP plasmid (5), which was used to generate OB-Fc, was digested with BamHI and NotI to remove the DNA fragment encoding the Fc protein. The pBMN-(OB-CAD-Fc)-I-GFP without the Fc fragment was then ligated with oligonucleotides (Supplementary Table S1) encoding 7 histidine residues, a termination codon, and BamHI and NotI restriction sites at its 5′ and 3′ ends, respectively. The resulting plasmid, pBMN-(Cad11-his7)-I-GFP, was used to generate retrovirus and transduce 293FT cell line as described previously (5). To purify Cad11-his protein, 293FT cells expressing Cad11-his were grown on 10 cm plates in DMEM supplemented with 10% FBS until 80% to 90% confluency. The serum-containing medium was collected after 2 days and concentrated 10 times in an Amicon Ultra-15 Centrifugal Filter (Millipore). Cad11-his fusion protein was purified by Ni-NTA agarose as described previously (6). Mice were immunized with Cad11-Fc protein and the monoclonal antibodies screened by ELISA assays using Cad11-his7-coated plates.

Fluorescence-activated cell sorting analysis

Fluorescence-activated cell sorting analyses (FACS) were conducted as described previously (4).

Intracardiac injection, bioluminescence imaging of mouse, and qPCR

The luciferase and GFP-expressing cells (PC3-mm2-Luc; 1 × 10⁶ cells/mouse) were mixed with control IgG (30 μg/mouse) or mAb 2C7 (30 μg/mouse) for 30 minutes before injecting into the left ventricle of male SCID mice. Mice were injected with antibodies immediately before tumor cell inoculation. Tumor growth was monitored weekly for 3 weeks using bioluminescence imaging. Images were acquired and analyzed with an IVIS Imaging System (Xenogen). Femurs and tibias were collected at the conclusion of the study and DNA was prepared using a Tissue and Blood DNA kit (Invitrogen). Quantitative PCR was conducted using oligonucleotides specific for human Alu sequence (Supplementary Table S1).

Generation of Cad11 extracellular domains for mAb epitope mapping

The extracellular domain DNA fragments EC1, EC2, EC3, and EC4, corresponding to the 1–159, 1–268, 1–383, 1–486 amino acid residues, respectively, of human Cad11 were cloned by PCR, confirmed by sequencing, and subcloned into pGEX4T-1 for the production of GST-tagged Cad11 proteins. A similar procedure was used to prepare EC3-containing serial deletions from its
C-terminal. Full-length Cad11 with mutations at tryptophan residues 2 and/or 4 (W2W4) or mAb 2C7 epitope were generated by overlapping PCR. The nucleotide sequences of primers used for deletions and mutagenesis are listed in Supplementary Table S1.

**Generation of L-cell lines stably expressing mutant Cad11**

Cad11 cDNA with mutations of the 6 amino acids in mAb 2C7 epitope to alanine (Cad11-6A) or mutations of the tryptophans at positions 2 and 4 to alanine (Cad11-W2A) were generated by overlapping PCR (see Supplementary Table S1). The mutant Cad11 cDNAs were cloned into the retroviral vector pBMN-I-neo, generated by replacing GFP cDNA in pBMN-I-GFP with that for the neo gene. L-cells infected with retroviruses were selected in G418-containing media.

**Statistical analyses**

Two-tailed, paired Student t test was used for the statistical analysis of the data. A P value of less than 0.05 was considered statistically significant. Data are expressed as the means ± SD.

**Results**

**Inhibition of Cad11-mediated cell aggregation by antibodies**

To examine whether antibodies could be used to block Cad11-mediated adhesion, an affinity-purified anti-Cad11 polyclonal antibody generated against the extracellular domain of Cad11 was examined for its effect on Cad11-mediated cell aggregation. An L-cell–based adhesion assay was established for identifying agents that inhibit cadherin-11–mediated cell–cell aggregation. L-cells are murine fibroblasts that do not adhere to one another as they do not express cell adhesion molecules. Thus, L-cells were transfected with control vector (L-vector) or Cad11 cDNA (L-Cad11). Cell aggregation was measured as a decrease in single cell numbers. As shown in Fig. 1A, expression of Cad11 led to cell aggregation, with a 70% decrease in single cell number after 3 hours. In contrast, the L-vector alone led to only an approximate 10% decrease in single cell number during the 3-hour assay (Fig. 1A).

At the concentration of 20 μg/mL, the polyclonal anti-Cad11 antibody exhibited a time- (Fig. 1B) and dose-dependent (Fig. 1C) inhibition of adhesion, whereas the control IgG did not affect the aggregation phenotype. Increasing the antibody concentration to 50 μg/mL did not result in further inhibition of cell aggregation (data not shown). In addition, the polyclonal anti-Cad11 antibody can achieve only a 50% maximal inhibition (Fig. 1C). It is possible that only a small fraction of the polyclonal antibody is able to block the aggregation. Nevertheless, these observations suggest that generation and selection for an antibody that blocks Cad11-mediated cell–cell aggregation may be used to determine the role of adhesion on bone metastasis.

Next, we generated Cad11-Fc and Cad11-his, that contain the extracellular domain of Cad11 fused with either Fc or 7-histidines. We used Cad11-Fc for immunization and Cad11-his for antibody screening. Among a panel of 21 anti-Cad11 mAbs generated, two mAbs, 1A5 and 2C7, inhibited aggregation by more than 70% (Fig. 1D).

**Characterization of anti-Cad11 mAb 2C7 and 1A5**

Using L-Cad11 cells in FACS analysis, mAb 2C7 showed an approximate 2-fold increased binding to Cad11 compared with that of mAb 1A5 (Fig. 2A). Similarly, mAb 2C7 exhibited stronger signals compared with mAb 1A5 when detecting Cad11 in L-Cad11 cells by Western blot analyses (Fig. 2B). Thus, we focused subsequent analyses using mAb 2C7. Next, we examined the specificity of mAb 2C7 for cadherins expressed in prostate cancer cells. C4-B4 cells express endogenous E-cadherin (Fig. 2C). By Western blot analysis, mAb 2C7 does not cross-react with E-cadherin. In contrast, PC3 prostate cancer cells and its subline, PC3-mm2 cells, express endogenous N-cadherin and Cad11. Anti-N-cadherin antibody reacted with proteins with molecular mass of 130 kDa, whereas mAb 2C7 reacted with a protein of 100 kDa (Fig. 2C), suggesting that mAb 2C7 does not cross-react with N-cadherin. On FACS analysis, mAb 2C7 reacted with C4-B4-Cad11 but not C4-B4-vector cells (Fig. 2D). mAb 2C7 also strongly reacted with PC3-mm2 cells (Fig. 2D). Downregulation of Cad11 in PC3-mm2 cells by shRNA (PC3-mm2/shCad11; ref. 4) significantly reduced mAb 2C7 binding to the cells (Fig. 2D). Similar results were observed with mAb 1A5 (data not shown). Together, these results suggest that mAb 2C7 and 1A5 specifically recognize endogenous Cad11 expressed in PC3 cells as well as exogenous Cad11 overexpressed in C4-B4 cells.

mAb 2C7 inhibits adhesion between PC3-mm2 and MC3T3-E1 osteoblasts

Next, we examined whether inhibition of Cad11-mediated adhesion by mAb 2C7 is sufficient to block the interactions between PC3-mm2 cells and osteoblasts. We first determined the dose of mAb required for inhibiting Cad11-mediated adhesion. In L-Cad11 cells, mAb 2C7 at a concentration of 1 μg/mL was sufficient to detectably inhibit cell aggregation, and a 50% inhibition was reached at 2 μg/mL (Fig. 3A, left), in contrast to the polyclonal antibody, which exhibits a maximum inhibition of 50% at 20 μg/mL (Fig. 1E). The maximum inhibition (80%) was reached at a dose of 10 μg/mL as increasing the amount of antibody to 20 μg/mL did not further increase the inhibition (Fig. 3A, right). Mouse IgG, used as a control, had no effect on cell–cell aggregation. These observations suggest that mAb 2C7 inhibits the Cad11-mediated aggregation efficiently. We further examined the effects of mAb 2C7 on the aggregation between PC3-mm2, a metastatic prostate cancer cell line that expresses endogenous Cad11, and MC3T3-E1, an immortalized osteoblast cell line. PC3-mm2 and MC3T3-E1 formed mixed aggregates in the presence of Ca2+ but not in Ca2+-free medium (Fig. 3B). Addition of mAb 2C7

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led to a significant inhibition of the Ca\textsuperscript{2+}-dependent PC3-mm2/MC3T3-E1 cell–cell aggregation (Fig. 3B).

**mAb 2C7 does not inhibit Cad11-mediated migration and invasion**

Our previous studies showed that expression of Cad11 also increases the migration and invasion, but not proliferation, of prostate cancer cells (4). We thus examined whether mAb 2C7 and 1A5 can affect prostate cancer cell migration or invasion. As shown in Supplementary Fig. S1, addition of mAbs 2C7 or 1A5 to C4-2B4/Cad11 cells did not affect cell migration or invasion. In contrast, mAb 1B9, which did not affect adhesion, was found to inhibit the migration (45%) and invasion (75%) of C4-2B4/Cad11 cells compared with those in controls (Supplementary Fig. S1A and S1B).

**Identification of mAb 2C7 binding domain in Cad11**

Next, we examined the mechanism by which mAb 2C7 inhibited Cad11-mediated cell–cell aggregation. Previous studies have shown the extracellular cadherin domain 1 (EC1) is responsible for the adhesive activity of cadherins, including E-, P-, and N-cadherin (7). Structural studies also showed that tryptophan 2 and 4 located at the N-terminus of EC1 mediates self-association through interaction with hydrophobic amino acids within EC1 (8). However, using atomic force microscopy, domains other than EC1 have been reported to be involved in cadherin11-mediated adhesion (9–12). mAb 2C7 could inhibit Cad11-mediated adhesion either by binding to the known adhesion motif located at EC1 or through a novel adhesion motif.
We first defined the domain to which mAb 2C7 epitope binds. We generated GST fusion proteins containing various lengths of Cad11 extracellular domain (Fig. 4A). Cad11 contains 5 extracellular cadherin repeats (EC), each approximately 110 amino acids long (13). The EC domain boundaries were derived from sequence alignment of the Cad11 amino acid sequence with that of other members of the cadherin family as described (13). Western blot analysis showed that mAb 2C7 and 1A5 did not bind to either GST-EC1 or GST-EC2 but reacted with GST-EC3 (Fig. 4A), suggesting that both mAb 2C7 and mAb 1A5 epitopes are localized within the EC3 domain of Cad11. We further refined the epitope within the EC3 domain. Five EC3 deletion mutants with sequential deletions of 20 amino acids from its C-terminus were generated. Western blot analysis of mAb 2C7 or 1A5 with these mutants showed that deletions of 40 amino acids from the EC3 C-terminus (EC3-40) led to a loss of signals with mAb 1A5 and a significant decrease in mAb 2C7 binding activity (Fig. 4B). These observations suggest that the mAb 2C7 and 1A5 epitopes recognize amino acids between 343 and 363 (Fig. 4B). EC3 mutants with sequential 5 amino acid deletions between amino acids 333–363 were then generated and the mAb 2C7 and 1A5 epitope was localized to amino acids 343–348 (Fig. 4C). Next, we generated alanine substitution mutants within the sequence YSLKVE corresponding to amino acid 341–348 and confirmed that amino acids 343–348 are critical for the binding of mAb 2C7 (Fig. 4D). Together, these results show that the mAb 2C7 epitope is localized to amino acids 343–348 within the EC3 domain of Cad11.
mAb 2C7 epitope is involved in Cad11-mediated adhesion

To examine whether the mAb 2C7 epitope is involved in Cad11 adhesion, we generated a Cad11 mutant with alanine substitutions in the YSLKVE sequence corresponding to amino acids 343–348 (Cad11-6A; Fig. 5A). Previous studies by Patel and colleagues (8) showed that the tryptophans located at the second and fourth amino acids of the N-terminus of Cad11 were essential for Cad11-mediated adhesion. Indeed, mutation of W2W4 to alanine abolished Cad11’s adhesion activity (8, 14). Thus, a Cad11 mutant with alanine substitutions at tryptophan 2 and 4 (Cad11-W2A) was also generated and used as a control (Fig. 5A). Both Cad11-6A and Cad11-W2A mutants were transfected into L-cells. Western blot analysis showed that wild-type Cad11 and Cad11-W2A, but not Cad11-6A, were recognized by mAb 2C7 (Fig. 5B). Both cadherin-11 mutant and wild-type proteins were recognized by mAb 5B2H5, which reacts with the cytoplasmic domain. Next, the expression of wild-type and Cad11 mutants were examined by immunofluorescence staining. Cell staining with mAb 5B2H5 showed membrane localization of wild-type and mutant Cad11 (Fig. 5C). To further examine whether the Cad11 mutants were targeted to the plasma membrane, cells were immunostained for FACS analyses. As shown in Fig. 5D, mAb 2C7 bound to wild-type and Cad11-W2A expressing cells with a mean intensity of 194 and 120, respectively, but much less to Cad11-6A, with a mean intensity of 43. Similar differences were observed when the median of intensity was used for comparison (Fig. 5D). The binding of mAb 1A5 to Cad11-6A was also significantly lower than that to wild-type Cad11, consistent with the epitope determination. In contrast with mAb 2C7 and 1A5, the binding of mAb 1B9, 1A1, or 4C1 to wild-type or Cad11 mutants did not show significant differences (Fig. 5D).

The ability of Cad11-6A to mediate cell aggregation was next examined. Cad11-6A did not show calcium-dependent cell aggregation (Fig. 5E). Cad11-WT and Cad11-W2A were used as positive and negative controls, respectively (8). A cell-to-substrate binding assay was used to further confirm the involvement of the mAb 2C7 epitope in Cad11-mediated adhesion. For these experiments, we used Cad11-Fc (5) to coat a 96-well plate. While Cad11-WT cells bound to plates coated with Cad11-Fc, Cad11-6A, and Cad11-W2A both exhibited binding similar to that observed with control vector-transfected cells (Fig. 5F). These studies identified a novel Cad11 adhesion motif that is necessary for Cad11-mediated adhesion. From these data, we conclude that mAb
mAb 2C7 inhibits the metastasis of PC3-mm2 cells to bone

To examine whether application of mAb 2C7 can inhibit prostate cancer cells metastasize to bone, an experimental metastasis model in which luciferase-labeled tumor cells were injected intracardially into SCID mice was used in our study. Mice were treated with one dose of mAb 2C7 or IgG (1.2 mg/kg, 30 μg/25g mouse), based on the antibody concentration determined from the study (Fig. 3A), through intraperitoneal injection. PC3-mm2-Luc cells were injected into the left ventricle of the mouse heart to disseminate tumor cells via the circulation. Bioluminescent imaging was conducted at 3, 8, and 15 days postinjection (Fig. 6A). The luminescence signals from the intracardiatic injection of PC3-mm2 cells showed that tumor cells were present not only in the femurs and/or tibias, but also the mandible areas, lymph nodes, and heart. Previous studies by us and several other groups established that the femur and tibia are the major metastasis sites from intracardiac injections of PC3-mm2-Luc cells (2), and hence we focused on these sites of experimental metastasis. Metastasis to other sites such as the mandible area appears to be a rodent-specific event and its relevance to human disease is not clear. In some mice, luminescence was observed in the chest area. This luminescence most likely occurred from leakage of PC3-mm2-Luc tumor cells.
Mutation of mAb 2C7 epitope on Cad11 abolished its homophilic adhesion activity. A, schematic presentation of Cad11 with mutations of the mAb 2C7 epitope to alanine (Cad11-6A) or mutations of two tryptophans at the N-terminus (Cad11-W2A). B, Western blot analysis of Cad11 mutants expressed in L-cells. Antibody 5B2H5 that recognizes Cad11 cytoplasmic domain was used as a positive control. C, immunofluorescence staining of L-cells expressing Cad11 mutants showed that they are all expressed on the plasma membrane. D, characterization of L-cells expressing Cad11 mutants by FACS analysis. Cad11-6A showed decreased binding with mAb 2C7 and mAb 1A5 but not with Cad11 mAbs 1B9, 1A1, or 4C1. E, cell aggregation assay showed that L-cells expressing Cad11-WT formed cell aggregates (*, P < 0.05), whereas Cad11-6A and Cad11-W2A could not form cell aggregates. F, cell-to-substrate assay showed that only Cad11-WT-expressing L-cells were able to bind to a Cad11-Fc-coated dish.
cells during injection of cells into the left ventricle. At Day 15, 4 of 5 mice in control group (IgG-treated) developed metastasis in the hind legs compared with those pretreated with control IgG. B, tumor volume in the hind legs was determined by bioluminescence and expressed as photons per second. Bioluminescence intensities from both ventral view and dorsal view of mice were determined and used as tumor volume. Left, average tumor volume in hind legs. Right, tumor volumes in individual hind legs. C, tumor volumes in the hind legs were determined by quantitative PCR using primers specific to human Alu sequence. Five mice were used in the control and mAb 2C7-treated group. Because of one and two mice in the control and treated group, respectively, dying before termination of the study, Alu qPCR was conducted on DNAs isolated from femur/tibia from 4 mice from the control group and 3 mice from the treated group. Thus, there are 8 values in control and 6 values in treated mice. Twenty nanograms of total DNA prepared from mouse hind legs was used for PCR. Number of tumor cells in bone, calculated on the basis of a standard curve derived from Alu PCR of PC3-mm2 DNA, is shown.

**Figure 6.** mAb 2C7 reduces PC3-mm2 metastasis to bone in a mouse experimental metastasis model. Luciferase-labeled PC3-mm2 cells were injected intracardially into SCID mice. A, bioluminescence images of mice. Mice pretreated with mAb 2C7 formed fewer skeletal metastases (red circles) in hind legs compared with those pretreated with control IgG. B, tumor volume in the hind legs was determined by bioluminescence and expressed as photons per second. Bioluminescence intensities from both ventral view and dorsal view of mice were determined and used as tumor volume. Left, average tumor volume in hind legs. Right, tumor volumes in individual hind legs. C, tumor volumes in the hind legs were determined by quantitative PCR using primers specific to human Alu sequence. Five mice were used in the control and mAb 2C7-treated group. Because of one and two mice in the control and treated group, respectively, dying before termination of the study, Alu qPCR was conducted on DNAs isolated from femur/tibia from 4 mice from the control group and 3 mice from the treated group. Thus, there are 8 values in control and 6 values in treated mice. Twenty nanograms of total DNA prepared from mouse hind legs was used for PCR. Number of tumor cells in bone, calculated on the basis of a standard curve derived from Alu PCR of PC3-mm2 DNA, is shown.
significantly reduced the number of tumor cells in bone. Taken together, these observations show that blocking Cad11-mediated adhesion is sufficient to reduce PC3-mm2 cells to metastasize to bone.

Discussion

Patients with advanced prostate cancer are at high risk of developing bone metastasis (1). Development of metastasis in bone has significant impact on the quality of life and survival of the patients (15, 16). Although it is obvious that therapies that prevent bone metastasis would improve the survival and quality of life for men with advanced prostate cancer, no such therapies exist that prevent prostate cancer bone metastasis. In this study, we developed two monoclonal antibodies that inhibit Cad11-mediated prostate cancer cell and osteoblast adhesion through binding a 6 amino acid motif in the extracellular domain (EC1) of the protein (8). From this point of view, antibodies that recognize the homophilic interaction motif should be among the most effective agents that block Cad11-mediated cell–cell adhesions. However, neither of the two most promising antibodies, i.e., mAb 2C7 and 1A5, selected from the 21 monoclonal antibodies against the extracellular domain of Cad11, recognizes the EC1 domain. This observation suggests the possibility that the previously defined homophilic interaction motif in EC1 of Cad11 may not be a good target region for developing therapeutic agents.

In addition to the well-established role of EC1 in cadherin-mediated cell–cell adhesion, there is evidence that additional sites in Cad11 are also involved in mediating homophilic interactions (12). Using atomic force microscopy, Pittet and colleagues (12) showed that Cad11-mediated adhesion strength is stronger than that in N-cadherin, suggesting that Cad11 may have a unique adhesion mechanism. Pittet and colleagues (12) further showed that Cad11 bonds are reinforced over time, with two distinct force increments. As a result, several possible models were proposed for the reinforcement of adherens junctions by Cad11. In the proposed adhesion model D (12), it was suggested that a combined strand-dimer as mediated by the EC1, and the EC interdigitation as mediated through EC1 to EC3, are involved in Cad11-mediated adhesion. Given that both mAb 2C7 and 1A5 recognize the 343–348 region of EC3, we suggest that this EC3 motif is at or within the suspected additional site in Cad11 that cooperates with EC1 in mediating homophilic interactions.

Although direct structural studies are required to define the role of the 343–348 motif of EC3 in the overall homophilic interaction of cell surface Cad11, our finding that both of the selected antibodies recognize this unique adhesion motif supports that this motif is a good target region for developing the second generation of more effective agents for Cad11-based antibody therapy. High affinity antibodies may be developed against this motif plus surrounding regions. In addition, the identification of this motif may also allow the development of peptidomimetic compounds that work similarly as antibodies in blocking Cad11-mediated cell–cell adhesion.

mAb 2C7-mediated decreased metastasis may result from multiple mechanisms. Inhibition of prostate cancer/osteoblast interaction and potentially prostate cell–cell interactions constitutes one of the mechanisms. Because we found that both the size of metastatic lesions in bone and the overall number of metastases are decreased when treated with mAb 2C7 (Fig. 6), this observation raises the possibility that other mechanisms may be involved. One possible mechanism is that the antibody therapy may inhibit outgrowth of metastases in bone, in addition to inhibiting prostate cancer targeting to bone. However, our previous studies have shown that knockdown of Cad11 did not have effects on PC-3 tumor growth in bone, based on in vitro proliferation assays (4). Another possibility is that mAb 2C7 may decrease metastasis by promoting cell killing through antibody-mediated mechanisms. Further studies will be required to address these possibilities.

One concern about antibody-based therapy is the potential undesirable toxicity resulting from the involvement of the targeted molecule in normal physiology. Cad11 has been shown to be involved in several developmental processes, including brain development (17–19). In adults, Cad11 is expressed at high levels in osteoblasts and low levels in brain, lung, and testis (13), indicating their continued functions in adult tissues. Importantly, targeted disruption of Cad11 did not affect mouse development except for a small reduction in bone density (20), suggesting that toxicities that might arise from anti-Cad11 therapies are likely to be minimal due to presence of functional redundancy.

Besides preventing bone metastasis, anti-Cad11 antibodies also have potential as therapeutics for rheumatoid arthritis and lung fibrosis. Cad11 is expressed in fibroblast-like synoviocytes (21) and is essential for the development of the synovium (22). In a mouse model, Lee and colleagues (22) showed that Cad11 is involved in rheumatoid arthritis, which was attenuated by an anti-mouse Cad11 antibody. Schneider and colleagues (23) showed that Cad11 contributed to pulmonary fibrosis, which could be treated by anti-Cad11–neutralizing monoclonal antibody. Whether these antibodies affect Cad11-mediated adhesion remains to be determined.

Prostate cancer cells and osteoblasts also express other cell adhesion molecules such as N-cadherin and integrins. It is likely that these adhesion molecules are also involved in the prostate cancer/osteoblast interactions. Tanaka and colleagues (24) reported that monoclonal antibodies targeting N-cadherin inhibits prostate cancer cell adhesion to...
fibronectin and also the migration, invasion, growth, metastasis and castration resistance of prostate cancer. The annexin II and annexin II receptor axis was also shown to regulate adhesion, migration, homing, and growth of prostate cancer (25). Although mAb 2C7 is able to elicit a significant decrease in bone metastasis in vivo, we predict that strategies targeting additional adhesion molecules or metastasis mediators will further enhance metastasis inhibition.

In summary, we showed that interfering with Cad11-mediated adhesion through a unique adhesion motif at EC3 domain is sufficient for inhibiting the metastasis of prostate cancer to bone. Further development of mAb 2C7 for human use is warranted.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
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References

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