Identification of a Novel Prostate Tumor Target, Mindin/RG-1, for Antibody-Based Radiotherapy of Prostate Cancer

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Abstract
Gene expression analysis showed that a human mindin homologue, mindin/RG-1, is expressed selectively in prostate tissues and that its expression level is elevated in some prostate tumors. Mindin/RG-1 protein expression is maintained in >80% of prostate cancers metastatic to bone or lymph nodes as well as in locally recurrent tumors in androgen-unresponsive patients. In contrast, mindin/RG-1 expression in other normal tissues is significantly lower than that seen in the prostate. A fully human antibody, 19G9, was generated against mindin/RG-1 protein and was shown to accumulate at high abundance in LNCaP tumor xenografts. Conjugates of this antibody with the chelator CHX-A9-DTPA were generated and radiolabeled with either 111In, 90Y, or 86Y. Small animal positron emission tomography imaging with the 86Y-radiolabeled conjugate showed very specific accumulation of the antibody in LNCaP tumor xenografts with clear tumor delineation apparent at 4 hours. The therapeutic efficacy of [99Y]-CHX-A9-DTPA-19G9 was evaluated in mice bearing LNCaP xenografts. A dose-finding study identified a nontoxic therapeutic dose to be ~75 µCi. Significant antitumor effects were seen with a single administration of radiolabeled antibody to animals bearing 200 to 400 mm3 tumors. Inhibition of tumor growth was observed in all treated animals over a 49-day period. At 49 days posttreatment, slow tumor growth resumed but this could be prevented for an additional 40-day period by a second administration of a 75 µCi dose at day 49. We conclude that [99Y]-CHX-A9-DTPA-19G9 is a novel antibody conjugate that has considerable promise for therapy of metastatic prostate cancer in androgen-unresponsive patients. (Cancer Res 2005; 65(18): 8397-405)

Introduction
Prostate cancer is one of the most prevalent cancers impacting men in the U.S. Although early stages of the disease can be cured, there is no effective therapy for late stages of the disease when the tumors become unresponsive to androgen-based therapies (1). Recently, genomics approaches, in particular gene expression profiling, have been used to identify new targets that could be applied for therapeutic purposes (2). To this end, we used a commercially available gene expression database to perform a screen for novel prostate-associated genes whose expression is maintained in tumors. Among a number of genes identified, we discovered a human mindin homologue, mindin/RG-1, that is abundantly expressed in the prostate and at higher levels in some prostate tumors. Mindin/RG-1 mRNA is expressed at significantly lower levels in a number of other tissues in the male. Mindins are members of the mindin/F-spondin family of secreted extracellular matrix proteins (3). They are characterized by the presence of two domains, FS1 and FS2 (F-spondin domains), together with a thrombospondin-type 1 repeat (TSP-1) domain. Members of this family include zebrafish mindin1 and mindin2, which accumulate in the basal lamina (4), Drosophila melanogaster M-spondin (5), rat (6), and human mindins (7). Rat mindin promotes adhesion and neuronal outgrowth of hippocampal embryonic neurons in vitro (6). Recently, a mouse mindin homologue was identified and found to be expressed in lymphoid tissue, in particular macrophages, and was shown to function as an opsonin for macrophage phagocytosis of bacteria (8). In this article, we show that the human mindin homologue, mindin/RG-1, is prostate-associated in humans and that its expression is maintained in primary prostate tumors, locally recurrent tumors from androgen-independent patients, and metastases to lymph node and bone tissues. The prostate-specific expression of mindin/RG-1 makes this extracellular matrix protein an attractive target for radioimmunotherapy, in which a radioisotope is coupled to antibodies recognizing mindin/RG-1 protein in prostate tumors and its metastases. We have generated a human antibody specifically recognizing mindin/RG-1 and show its selective accumulation in human prostate cancer xenografts and its pronounced efficacy after radiolabeling.

Materials and Methods
Mindin/RG-1 mRNA analysis. Total RNA was isolated from prostate and prostate tumor tissue using the RNeasy mini kit (Qiagen, Valencia, CA) according to the manufacturer’s recommendations. Normal tissue total RNA was purchased from Clontech (Palo Alto, CA). Tumor tissue total RNA was purchased from Biochain (Hayward, CA). A real-time quantitative reverse transcription-PCR assay was developed to measure mindin/RG-1 using specific primers and probes: 5′-GCAGCACACAGCTGACTACAG-3′ (forward primer), 5′-TCACACTGCTAGGAGGTCGTAG-3′ (reverse primer), 5′-TGTCGAGAATGATGCAGAAGC-3′ (probe). For Northern blot analysis, 32P-radiolabeled cDNA probes were prepared with a Rediprime II random prime labeling system (Amersham, Piscataway, NJ) using a 996 bp human mindin/RG-1 cDNA as template on human multiple tissue Northern blots (BD Biosciences/Clontech, Mountain View, CA). Blots were washed at high stringency and exposed to X-Omat MR film (Kodak, Rochester, NY) for various times.

Generation of mindin/RG-1 protein expressed in baby hamster kidney cells. A cDNA with the mindin/RG-1 coding region was obtained
Mindin/\(RG-1\) protein purification. (a) Untagged mindin/\(RG-1\) protein was purified from serum-free medium from BHK cells expressing mindin/\(RG-1\) protein by diluting the medium 1:1 in 20 mmol/L sodium acetate buffer (pH 6.5) and loading to a 5 ml Q-Sepharose ion exchange column. Fractions were monitored by Western blot, and those containing mindin/\(RG-1\) were pooled and further purified using a SuperDex-75 size exclusion column. (b) Mindin/\(RG-1\) protein containing the 6-histidine expression tag was purified from BHK cell conditioned medium using NINTA-Sepharose affinity chromatography. Peak fractions were pooled and dialyzed into PBS and stored at \(-80^\circ C\).

Generation, cloning and expression of full length human anti-mindin/\(RG-1\) antibodies in Chinese hamster ovary cells. Antibodies were originally produced by hybridomas using Medarex's UltiMaB Human Antibody Development System (Medarex, Inc., Milpitas, CA). For Chinese hamster ovary (CHO) expression, the human antibody 19G9 VH and VL sequences were cloned into an expression vector pIE-Sr y1a [pIE] (Medarex) and transfected into DGG4 CHO cells. Transfection was carried out with LipofectAMINE-2000 following the manufacturer's instruction. Selective conditions were applied at ~48 hours posttransfection. Co-selec- tion for G418 and dihydrofolate reductase was first carried out with \(\alpha\)-MEM supplemented with 5% heat-inactivated dialyzed FCS, 400 \(\mu\)g/mL of G418, and 20 mmol/L of methotrexate in 96-well plates. A series of antibody-expressing CHO cell clones were selected and subjected to further amplification in iteratively increasing concentrations of methotrexate. The final selection were based on quantification using analytic protein A chromatography and Amersham absorbance.

Immunoreactivity of anti-mindin/\(RG-1\) antibodies. The immunore-a ctivity was tested by (a) 6-His Capture ELISA, (b) Biacore analysis using BHK expressed protein, and (c) RIA. Kinetic analysis of binding affinity of purified antibodies to native mindin/\(RG-1\) was done using a method in which soluble BHK-mindin/\(RG-1\) was captured by immobilized monoclonal antibody. Anti-human Fc antibody was covalently attached to the Biacore sensor chip via amine coupling. Association and dissociation data were fit to a 1:1 Langmuir model.

Immunohistochemistry. Frozen and paraffin-embedded tissue samples were obtained from PhenoPath (Seattle, WA) and from the pathology department at Innsbruck, Austria. Low-density human tissue arrays were obtained from Innomegen (San Ramon, CA; cat. TS-4201-05). Binding of the primary antibodies (RG-1/3c; 19G9) was visualized using a Streptavidin kit (Zymed, San Francisco, CA) with 3,3'-diaminobenzidine as chromogen.

In vivo characterization of human anti-mindin/\(RG-1\) antibodies. Male, nude (nu/nu) mice, 6 to 8 weeks of age were obtained from Simonsen Laboratories (Gilroy, CA). Mice were maintained in facilities accredited by the American Association for Accreditation of Laboratory Animal Care, and all experiments were conducted in accordance with principles and procedures approved by the Animal Care and Use Committees at Berkeley Biosciences and Washington University. Mice were implanted s.c. with LNCaP cells (102 cells/mouse). Approximately 4 to 6 weeks after receiving the cell implant, mice bearing tumors of 50 to 400 mm3 were selected and randomized into groups (\(n = 15\) for biodistribution, \(n = 20\) for efficacy studies). Antibodies were radiolabeled with \(111\)In for biodistribution studies, \(\text{\(^{198}Y\)}}\) for positron emission tomography (PET) studies, or \(\text{\(^{58}Y\)}}\) for efficacy studies and given i.v. through the lateral tail vein.

Biodistribution studies. At selected time points (days 1, 3, 6, and 9 postinjection) animals treated with [\(\text{\(^{111}In\)\)-CHX-A\(\text{-DTPA}\)] were randomized to separate treatment groups (antibody alone, radiolabeled human IgG control antibody ([\(\text{\(^{111}In\)\)-CHX-A\(\text{-DTPA}\)])), and [\(\text{\(^{198}Y\)\)\]-CHX-A\(\text{-DTPA}\)]). Exsanguination was performed for blood sampling and anesthesia. All organs, including blood and tumor, were collected, weighed and radioactivity was measured in a gamma counter (Auto-Gamma Cohra II, Packard, Downers Grove, IL) and expressed as the percentage of injected dose per gram (% ID/g).

Positron emission tomography imaging studies. The \(\text{\(^{198}Y\)}}\) was produced on the CS15 cyclotron by the \(\text{\(^{86}Sr(p,n)\text{\(^{86}Y\)}}\) reaction (10). The imaging of \(\text{\(^{198}Y\)\)-CHX-A\(\text{-DTPA}\)} was done at Washington University on the micro-PET-R4 tomograph (Concorde Microsystems, Inc., Knoxville, TN). Coregistra-tion of the PET images was achieved in combination with a micro-CAT-II camera (Inteck Inc., Knoxville, TN). The image registration between micro-CT and PET images was accomplished using a landmark registration tech-nique AMIRA image display software (AMIRA, TGS Inc., San Diego, CA).

In vivo efficacy studies. LNCaP-bearing nude mice (50-400 mm3) were randomized (\(n = 20\)) to different treatment and control groups (antibody alone, radiolabeled human, isotype-matched IgG control antibody, or no treatment). Tumor volumes and general toxicity as measured by body weight and general clinical appearance was monitored for up to 30 days (study 1) or 90 days (study 2). In study 1, \(\text{\(^{198}Y\)\)-CHX-A\(\text{-DTPA}\)} was given at a single i.v. dose of 25, 50, 75, or 100 \(\mu\)g. In study 2, tumor-bearing animals were given a single i.v. dose of 75 pCi of \(\text{\(^{198}Y\)\)-CHX-A\(\text{-DTPA}\)} at day 0 and a second dose of 75 pCi at day 49.

Serum prostate-specific antigen determination. Human prostate-specific antigen (PSA) was quantified in the plasma from LNCaP-bearing mice by an ELISA kit from Diagnostic Systems Laboratories, Inc., (Webster, TX) and read on a Molecular Devices Versamax microplate reader with SoftMax Pro software (Sunnyvale, CA).

Statistical analysis. Values from biodistribution studies are averages of three individual mice \(\pm SE\). For the animal efficacy studies, the effect of treatment on weekly tumor volumes and final tumor weights was analyzed by nonparametric tests and deemed significant if \(P < 0.05\). The overall effect of treatment was assessed by the Kruskal-Wallis test, whereas comparison between two treatment groups was analyzed by the Mann-Whitney U test. Reported \(P\) values are from the Mann-Whitney U test.

Results

Mindin/\(RG-1\) mRNA is highly expressed in prostate tissue, both normal and tumor. Mindin/\(RG-1\) was initially identified as one of several human genes expressed in high abundance in prostate tissue libraries by mining Incyte's LifeSeq database. The tissue-specific expression of mindin/\(RG-1\) was confirmed by reverse transcription-PCR–based analysis using TaqMan probes and RNA obtained from clinical samples. mRNA for mindin/\(RG-1\) was expressed predominantly in the prostate (both tumor and normal samples), and at significantly lower levels in other tissues (Fig. 1A). A similar picture was seen by Northern blot analysis using a mindin/\(RG-1\)–specific probe. A message of ~1.9 kb was detected at significantly higher levels in the prostate samples as compared with any RNAs from other tissues (Fig. 1B). In this set of tissue samples, mindin/\(RG-1\) mRNA was expressed at 8-fold higher abundance in the prostate than any other tissue sample. A band with a significantly lower intensity at about 6 kb was also seen in the Northern blot and this may represent an alternatively spliced form of mindin/\(RG-1\) mRNA. Furthermore, we have determined that mindin/\(RG-1\) gene maps to chromosome 4p16.3 and the dominant mRNA is coded by six exons (data not shown).

Mindin/\(RG-1\) protein is expressed in prostate tissue, both normal and tumor, and in metastases to lymph node and bone. Mindin/\(RG-1\) mRNA encodes for a protein containing 331
mindin/RG-1 expression was monitored in human prostate tissue samples, both normal and cancerous, and in metastases from prostate tumors to lymph node and bone, using immunohistochemistry procedures. The mindin/RG-1 protein was expressed in ~80% of primary prostate tumors (15 of 18) and in metastases to lymph node (8 of 10) and bone (18 of 23; Table 1). As most patients that present with advanced disease have tumors that are unresponsive to androgen ablation therapy, it was of interest to determine if mindin/RG-1 expression was maintained in tumors from this group of patients. As shown in Table 1, ~80% of local recurrent tumors from androgen-independent patients (22 of 26) maintain expression of mindin/RG-1 protein. Thus, expression of mindin/RG-1 is seen in the majority of tumors from patients that are androgen-unresponsive as well as from patients that have early stage disease.

Expression of mindin/RG-1 was also studied in a large set of formalin-fixed, paraffin-embedded normal tissues. Specific staining was seen in normal prostate tissue as monitored by antibody RG-1/3c and was confirmed in frozen normal prostate tissue using biotinylated human antibody 19G9 (data not shown). Normal prostate exhibited moderate to strong staining with significant variability from sample to sample. Granulocytes infiltrating tissue sections were also found to stain strongly in some tissue sections.

**Fully human antibodies against mindin/RG-1 show nanomolar affinity to mindin/RG-1 protein.** To evaluate the option of targeting mindin/RG-1 for antibody-based therapy, mindin/RG-1 protein was expressed in BHK cells, purified as described in Materials and Methods, and used to immunize transgenic mice using Medarex’s UltiMAB® technology. Following immunization, hybridomas were generated and culture supernatants from the

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**Figure 1.** Mindin/RG-1 mRNA is overexpressed in human prostate tissue as shown by (A) TaqMan and (B) Northern blot analysis. A. RNA samples were prepared from the indicated normal tissues and from prostate tumors as described in Materials and Methods and used for TaqMan analysis of mindin/RG-1 mRNA. Levels were normalized to 18S rRNA levels. Mindin/RG-1 is expressed primarily in the prostate tissues, both normal (lanes 15-22) and tumor (lanes 23-30), and at significantly lower levels in other normal tissues: bone marrow (lane 1), spleen (lane 2), spinal cord (lane 3), small intestine (lane 4), thymus (lane 5), pancreas (lane 6), colon (lane 7), lung (lane 8), stomach (lane 9), skeletal muscle (lane 10), brain (lane 11), heart (lane 12), kidney (lane 13), liver (lane 14); human prostate tumor cell lines: PC-3 (lane 31), LNCaP (lane 32). B. mindin/RG-1 mRNA was identified as a 1.9 kb message in different human tissues with prostate tissue (lane 3) being the organ of highest expression when compared with other human tissues such as spleen (lane 1), thymus (lane 2), testis (lane 4), ovary (lane 5), small intestine (lane 6), colon (lane 7), peripheral blood (lane 8), heart (lane 9), brain (lane 10), placenta (lane 11), lung (lane 12), liver (lane 13), skeletal muscle (lane 14), kidney (lane 15), and pancreas (lane 16). C, the levels of mindin/RG-1 mRNA were quantified by scanning and expressed as relative levels on pixel scale, confirming the overexpression of mindin/RG-1 mRNA in prostate tissue.
hybridomas were screened by a capture ELISA assay to identify antibodies recognizing the native protein. Several monoclonal antibodies were identified and four were further characterized by using BIACore technology to determine kinetic variables. These four antibodies exhibited affinities in the nanomolar range (8.4–34 nmol/L). As an example, the IgG1 19G9 (K\text{d} 8.4 nmol/L) bound to native but not denatured protein and detected mindin/RG-1 protein by Western blot analysis only on nonreducing gels (data not shown). Antibody 19G9 was cloned and expressed in CHO cells, and purified from the culture medium by Protein A chromatography. The CHO-expressed antibody and the hybridoma-produced antibody exhibited virtually identical affinity for mindin/RG-1 (data not shown).

19G9 antibodies can be conjugated to CHX-A$^{00}$-DTPA and radiolabeled with $^{111}$In, $^{90}$Y, or $^{86}$Y. The chelator CHX-A$^{00}$-DTPA was conjugated to the 19G9 antibody produced by CHO cells and to a control human IgG1 antibody through primary amine groups on antibody lysines as previously described (11). Typically, specific activities ranged from 0.25 to 1.0 mCi/mg. Specific activities of 8 mCi/mg were also experimentally achieved. A molar ratio of 3:1 (chelator to antibody) was achieved as determined by two methods: (a) a spectrophotometric method using Arsenazo III (12) or (b) a titration method using $^{111}$In (13). The absence of antibody aggregates in the conjugate preparation was confirmed by size exclusion chromatography (data not shown). The CHX-A$^{00}$-DTPA-19G9 antibody conjugate was radiolabeled with $^{111}$In for biodistribution studies, $^{86}$Y for small animal PET imaging studies, or $^{90}$Y for in vivo efficacy studies. The radiochemical yield was always in the order of 70% to 95%, and the radiochemical purity as determined by thin layer chromatography (11), was generally >97% and frequently >99%. It was shown by ELISA and RIA that the immunoreactivity of the antibody was maintained after conjugation and after radiolabeling, demonstrating binding of the antibody 19G9 to mindin/RG-1 with nanomolar affinity (data not shown).

Radiolabeled CHX-A$^{00}$-DTPA-19G9 antibody shows a high and tumor-specific accumulation in a human prostate tumor (LNCaP) xenograft as determined in biodistribution and small animal positron emission tomography imaging studies. In order to monitor the tumor-specific accumulation of 19G9 in vivo, biodistribution studies were done using $^{111}$In-labeled CHX-A$^{00}$-DTPA-19G9 and an $^{111}$In-labeled isotype-matched human antibody that does not bind to mindin/RG-1 (CHX-A$^{00}$-DTPA-IgG1), thus serving as a negative control. As shown in Table 2, $^{111}$In-CHX-A$^{00}$-DTPA-19G9 showed a high and target-specific accumulation in the LNCaP xenograft, whereas no specific tumor accumulation was seen with the radiolabeled control antibody, CHX-A$^{00}$-DTPA-IgG1. In order to visualize the tumor accumulation in LNCaP tumor–bearing mice, small animal PET studies were done using CHX-A$^{00}$-DTPA-19G9 labeled with $^{86}$Y as a PET tracer. All animals ($n$ = 4) showed high levels of activity in the blood at 1 hour, which rapidly cleared after 4 hours. At 4 hours, LNCaP tumors could already be delineated in all subjects. Over the 72-hour imaging period, exceptionally high tumor to background contrast was noted in all animals, demonstrating the excellent targeting properties of radiolabeled 19G9 antibody recognizing mindin/RG-1 protein (Fig. 4).

$^{90}$Y-CHX-A$^{00}$-DTPA-19G9 shows a dose-dependent, antibody-specific antitumor effect in a human prostate tumor (LNCaP) xenograft after a single or double i.v. injection. To determine an efficacious, but nontoxic, dose of the radiolabeled antibody 19G9 in an animal model of human prostate cancer, various doses (25, 50, 75, and 100 \(\mu\)Ci) of $^{90}$Y-CHX-A$^{00}$-DTPA-19G9 were injected i.v. into tumor (LNCaP)–bearing nude mice ($n$ = 20; tumor volume, 95–425 mm$^3$). An additional group was administered unlabeled antibody; a “no treatment” control group was also

![Figure 2. Sequence alignment of human, mouse, and rat mindin/RG-1 homologues.](https://cancerres.aacrjournals.org)
DTPA-19G9 that was statistically significant for all doses >25 µCi when compared with the control groups. All doses were well-tolerated (body weight loss <10%) and no deaths occurred at any dose tested. As a secondary measure of tumor burden, serum PSA levels were assessed on day 27. PSA is widely used in the clinic as a screening tool to either determine the presence of prostate cancer or to monitor therapeutic effects in prostate cancer patients. LNCaP tumors secrete PSA, which in xenograft models, could be used as an additional biomarker for tumor burden. As shown in Fig. 5, the decrease of PSA levels correlates very well with the antitumor effect of [90Y]-CHX-A-DTPA-19G9. These efficacy data were extended in a second study, in which 75 µCi of [90Y]-CHX-A-DTPA-19G9 or a nonspecific, human IgG1 control antibody ([90Y]-CHX-A-DTPA-IgG1) were injected i.v. into tumor-bearing (LNCaP) nude mice (n = 20; tumor volume, 50–400 mm³). An additional control group did not receive any treatment. Tumor growth was monitored by caliper measurements and treatment-related toxicity was evaluated by body weight change and general clinical appearance. A significant antitumor effect without treatment-related toxicity was observed after a single i.v. injection of [90Y]-CHX-A-DTPA-19G9 (Fig. 6). In contrast, animals receiving no treatment or 75 µCi of the control antibody exhibited rapid tumor growth and needed to be euthanized on day 45 due to rapid tumor growth. On day 49, the 20 surviving mice were divided into two subgroups: one subgroup (n = 10) received a second i.v. injection of 75 µCi Y-90-labeled CHX-A-DTPA, whereas the remaining group (n = 10) received no further treatment. These two subgroups were monitored for body weights, tumor volume, and animal deaths for an additional 6 weeks. As shown in Fig. 6A, whereas regrowth of the tumors was observed for the animals that did not receive a second treatment of radiolabeled antibody, a significant antitumor effect, without any treatment-related toxicity as monitored by body weight loss and animal death (Fig. 6B), could be seen for at least 90 days in animals receiving the second administration of antibody.

**Discussion**

Using various gene expression analysis tools, as well as immunohistochemical techniques, we have shown that mindin/RG-1 mRNA and protein are expressed in prostate tissue at significantly higher levels than found in other tissues in the male. mRNA for mindin/RG-1 is detectable in tissues other than the prostate by both TaqMan analysis and by Northern blot analysis but levels are significantly lower than that seen in prostate tumor samples. Immunohistochemical studies, however, failed to show specific staining for mindin/RG-1 protein in non-prostate normal tissue sections. These differences may be a result of the different sensitivities of the procedures used and/or may reflect a low level of translation of mindin/RG-1 mRNA in tissues other than the prostate.

The tissue distribution of mRNA for mindin/RG-1 in the human seems to be significantly different from that reported for mouse mindin, where expression of mindin is seen in the lung, lymphoid tissues, and heart as well as a number of other tissues (data not shown, ref. 8). It has been reported that mindin is expressed in mouse macrophages and that it may function as an opsonin for clearance of bacteria (8). Our preliminary analysis of mindin/RG-1 expression in human macrophages by Western blot has revealed that mindin/RG-1 is expressed in differentiated macrophages but not in circulating monocytes (data not shown). It is also notable that immunohistochemistry studies revealed expression in granulocytes infiltrating tumor tissue.

The abundant expression of mindin/RG-1 in the prostate in humans prompts the question of what its role might be in normal prostate tissue as well as in prostate tumors. In the rat, a mindin homologue has been reported to promote outgrowth of embryonic hippocampal neurons (6). In the mouse, a mindin homologue has been reported to be a pattern-recognition molecule for microbial pathogens (8). Although there is no information regarding its function in humans, it is likely that the role of mindin/RG-1 is distinct from any opsonizing role that the protein may have. Such alternate roles for extracellular matrix molecules in modulation of cell attachment, growth, and differentiation have been documented previously for other extracellular matrix
molecules, and it is possible that mindin homologues have similar properties (14).

Mindin/RG-1 protein is abundantly expressed in both normal and tumor tissues with expression in tumors being generally higher than that in normal prostate. Significantly, expression of mindin/RG-1 is maintained in tumor metastases to distal sites including the bone and lymph nodes. Its expression is also maintained in recurrent tumors from patients that are unresponsive to androgen ablation therapy. The distribution of the protein, taken together with the fact that it seems to be secreted into the extracellular milieu, makes it a promising target for antibody based therapy of prostate cancer. This is supported by our studies with the mindin/RG-1 specific antibody 19G9, which was shown by biodistribution studies using \([^{111}\text{In}]\)-CHX-A\(^{2-}\)-DTPA-19G9 as well as PET imaging studies using \([^{86}\text{Y}]\)-CHX-A\(^{2-}\)-DTPA-19G9 to accumulate specifically and at high levels in LNCaP tumor xenografts in nude mice (15). The accumulation was target-specific as shown by the absence of accumulation of control antibodies in the tumors. These results were confirmed with a second mindin/RG-1–specific antibody, 34E1 (data not shown). It is important to note that neither 19G9 nor 34E1 bind to mouse protein, and consequently it was possible to conduct the biodistribution studies without concern for the tissue distribution of the mouse homologue, which is quite distinct from that of the human homologue.

The chelator CHX-A\(^{2-}\)-DTPA exhibits high affinity for several isotopes that are suitable for either diagnostic or therapeutic purposes, including \[^{111}\text{In}, {^{90}}\text{Y}, {^{99}}\text{Y}, and {^{177}}\text{Lu}\). PET imaging studies with the positron emitter \[^{90}\text{Y}\] showed the ability of the mindin/RG-1–specific antibodies to delineate the LNCaP tumor with a high tumor to background ratio. Our therapeutic efficacy studies were done with \[^{90}\text{Y}\], a pure \(\beta\)-emitter. Highly significant antitumor effects

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<th>Tissue source</th>
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<tr>
<td>Lymph node metastases</td>
<td>8 out of 10</td>
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<tr>
<td>Bone metastases</td>
<td>18 out of 23</td>
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<tr>
<td>Locally recurrent tumors in androgen insensitive patients</td>
<td>22 out of 26</td>
<td>moderate 2+ heterogeneous staining</td>
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NOTE: Immunohistochemical studies were done using the polyclonal rabbit antibody RG-1/3c on formalin-fixed, paraffin-embedded tissues. Scoring: 1+, faint staining or localized staining; 2+, moderate focal staining of majority of tumor specimen; 3+, strong focal staining of majority of tumor specimen.

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</table>

NOTE: The calculated percentage of injected dose per gram (% ID/g) for \([^{111}\text{In}]\)-labeled 19G9 and isotype-matched control antibody IgG\(_1\) at 1, 3, 6, and 9 days after i.v. injection into LNCaP tumor–bearing nude male mice is shown as the mean of three mice ± SE. \([^{111}\text{In}]\)-CHX-A\(^{2-}\)-DTPA-19G9 shows a high and tumor-specific accumulation when compared with a nonspecific \([^{111}\text{In}]\)-CHX-A\(^{2-}\)-DTPA-control IgG.
were seen with 19G9 over a dose range of 25 to 100 μCi/mouse with inhibition of tumor growth being observed at all doses above 25 μCi. At doses of 75 μCi per mouse, complete inhibition of tumor growth was seen for up to 50 days, although residual tumor cells were still present at sacrifice. Significantly, at the 75 μCi dose, no long-term toxicity of the radiolabeled conjugate was detected as monitored by loss of weight, demonstrating that a clear therapeutic window can be established where tumor growth inhibition can be achieved without significant toxicity. Similar data was obtained with a second antibody, 34E1 (data not shown), supporting the suitability of mindin/RG-1 as a therapeutic target.

Forty-nine days after administration of a single dose of radiolabeled antibody, regrowth of the tumors became apparent. This, however, could be prevented by administration of a second dose of radiolabeled antibody at this time. Following administration of this second dose, no regrowth of tumors was detectable over the next 41 days of the study. When the study was terminated on day 90, all mice were alive bearing either no detectable tumor or small tumor masses at the site of implantation. Histologic analysis of the smallest of these tumors revealed variably sized necrotic regions inter-spacing small clusters of viable tumor cells. Often, collagen scarring and hemosiderin-ladened macrophages were present around the tumor cells.

The antitumor effects were also clearly shown by quantitation of PSA levels in the sera of mice. Highly significant reductions in the levels of circulating PSA were seen in mice treated with radiolabeled antibodies. The mean levels of PSA did not return to zero, consistent with the presence of some viable cells in the remaining small tumor masses. However, when the levels were evaluated on an animal by animal basis, some mice did not have any or extremely low levels of PSA, demonstrating that in some mice, virtual eradication of tumor may have occurred.

Several other cell surface or secreted proteins have been suggested as possible targets for immunotherapy of prostate cancer. These include PSMA (16), PSCA (17), TMEFF2 (18), protein (19), STEAP2 (20), PSGR (21), Trp-p8 (22), and NGEP (23). Mindin/RG-1 is distinct from these in lacking a transmembrane domain and is secreted from tumor cells but can also loosely associate with the outer surface of the plasma membrane. Although this location precludes its use as a target for antibody-drug conjugate–based therapies that require internalization of antibody-drug conjugates, mindin/RG-1 is clearly an attractive target for therapeutic approaches which do not require internalization of the antibody conjugate, e.g., radioimmunotherapy. The location of the target at the cell periphery and the high expression level may provide increased access of antibodies to the target when compared with strictly membrane-associated proteins. Radiolabeled antibodies provide a significant bystander killing effect that may be extremely important in eradicating tumors where target expression may be heterogeneous throughout the tumor or tumor metastases. Whereas the location of mindin/RG-1 may be advantageous with respect to antibody access, we recognize that some protein may be shed from the tumor into the serum. Our preliminary analysis indicates that the levels of mindin/RG-1 in serum are low. However, more extensive analysis of this will be necessary to gain a better understanding of potential toxicities that could result from antibody binding to protein in the serum. Clearly, no evidence of systemic toxicity was seen in the animal model studies reported here.

Of the previously mentioned targets, most have been evaluated as targets for drug-based immunotherapy, e.g., maytansinoid-conjugated antibodies targeting PSCA or PSMA (17, 24) or Auristatin E-conjugates of antibodies targeting TMEFF (25). Recently, the use of TMEFF2/Tomoregulin targeted radioimmunotherapy...
A radioimmunotherapy approach to prostate cancer is being pursued in the clinic with [90Y]-labeled J591, an anti-PSMA antibody conjugated to 1,4,7,10-tetra-azacyclododecane N,N\[d\],N\[-V-J-tetraacetic (DOTA) as the chelator (27). Preclinical studies with [90Y]-DOTA-J591 have been reported in LNCaP cells propagated as xenografts in nude mice in a fashion similar to that described here. Although antitumor effects were reported, it was found that efficacy was achieved in this model only in conjunction with significant toxicity and animal death (28, 29). In contrast, the therapeutic window that we report here with [90Y]-CHX-A00-DTPA-19G9 is clearly superior to that reported for [90Y]-DOTA-J591 in the same xenograft model. Because signs of efficacy were observed with [90Y]-labeled J591 in the clinic only at or above the maximal tolerated dose (27), the large therapeutic window observed with our mindin/RG-1–specific antibody may translate into the much needed improved efficacy and tolerability in the radioimmunotherapy of advanced prostate cancer.

Figure 5. Antitumor effect of \(^{90}\text{Y}\)-CHX-A\(^\cdot\)DTPA-19G9 in a human prostate tumor xenograft is dose-dependent and correlates with PSA levels. 
A, progress of tumor growth (n = 20). B, tumor weights on day 33 means (n = 19). LNCaP tumor xenografts were established s.c. in male athymic mice. Treatment was started on day 0 when tumors reached a volume of 95 to 425 mm\(^3\). Tumor-bearing animals were evenly distributed to treatment and control groups. Protein concentration in all groups was normalized to the same antibody concentration. \(^{90}\text{Y}\)-CHX-A\(^\cdot\)DTPA-19G9 was given at a single i.v. dose of 25, 50, 75, or 100 μCi. Control groups included a group receiving unlabeled antibody alone (0 μCi group) and a no-treatment group. Tumor growth inhibition of \(^{90}\text{Y}\)-CHX-A\(^\cdot\)DTPA-19G9 occurred at all doses of \(^{90}\text{Y}\) >25 μCi, with the greatest effect seen at 75 and 100 μCi (P < 0.05, Mann-Whitney, comparison between all groups). Columns, means; bars, ± SE. 
C, animals (n = 17-19) were bled for serum PSA measurements via ELISA 27 days after treatment. Effect of \(^{90}\text{Y}\)-CHX-A\(^\cdot\)DTPA-19G9 treatment on circulating PSA levels correlates with tumor burden and was statistically significant (P < 0.05, Mann-Whitney, comparison versus no treatment). Columns, means; bars, ± SE.
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References

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