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

Renate Parry,1 Doug Schneider,1 Debra Hudson,2 Debbie Parkes,1 Jian-Ai Xuan,1 Alicia Newton,1 Pam Toy,1 Rick Lin,1 Rick Harkins,1 Bruno Alickie,1 Sandra Biroc,1 Peter J. Kretschmer,1 Meredith Halks-Miller,1 Helmut Klocner,1 Ying Zhu,1 Brent Larsen,1 Ronald R. Cobb,1 Peter Bringmann,1 Georg Roth,1 Jason S. Lewis,1 Harald Dinter,1 and Gordon Parry1

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-A⁰-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 [⁹⁰Y]-CHX-A⁰-DTPA-19G9 was evaluated in mice bearing LNCaP tumor 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 occurred 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 [⁹⁰Y]-CHX-A⁰-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

Mиндin/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'-GCAGCAACAGCTGCTGACAG-3' (forward primer), 5'-TCACCCGCTCAGCCTACG-3' (reverse primer), 5'-TGTGCCGAAATGTAGTCTCAGCA-3' (probe). For Northern blot analysis, ²⁵P-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.

Requests for reprints: Renate Parry, Berlex Biosciences, 2600 Hilltop Drive, Richmond, CA 94806. Phone: 510-669-4734; Fax: 510-669-4270; E-mail: renate_parry@berlex.com.

©2005 American Association for Cancer Research.
doi:10.1158/0008-5472.CAN-05-1203
from Incyte (Palo Alto, CA). The coding sequence was PCR-amplified with forward (5'-TCCCTTCTAGGACCATGGAAGACCCAGGCCGCC-3') and reverse (5'-AAGGCATCACTGTTAGACCGTGTTACCAGGGAC-3') primers for 35 cycles (annealing temperature 60°C, extension time of 120 s) with Pfu Turbo polymerase (Clontech), and ligated into pSIN rep21 (9). Similarly, a plasmid containing full-length mindin/RG-1 with GSS-6xHis added to the carboxyl terminal (pPEG-9) was obtained using the same PCR forward primer, with the reverse primer being 5'-AGTAAAGGCTACATCTAGTGTAAGGTGTGATGCTGCTGCC- GCCGACCGCTATCCGAGGCCAC-3'. Expression plasmids pPEG6, for the carboxyl-terminal 6xHis-tagged mindin/RG-1, and pPEG8, for the untagged mindin/RG-1, were transfected into baby hamster kidney (BHK)-21 cells (American Type Culture Collection, Rockville, MD) using LipofectAMINE-2000 (Invitrogen, Carlsbad, CA). Expression of mindin/RG-1 protein into the culture medium was monitored by Western blot analysis using either an anti-6xHis antibody or a rabbit polyclonal anti-mindin/RG-1 antibody plus horseradish peroxidase–labeled second antibody.

**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 mM NaCl, 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 NIN TA-Sepharose affinity chromatography. Peak fractions were pooled and dialyzed into PBS and stored at −80°C.

**Generation, cloning and expression of fully 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 I9G9 VH and VL sequences were cloned into an expression vector pIE-Sr into an expression vector pIE-Sr (21) and expressed as the percentage of injected dose per gram (% ID/g).

**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, isotope-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, [186Y]-CHX-A-DTPA-IgG was given at a single i.v. dose of 25, 50, 75, or 100 μCi. In study 2, tumor-bearing animals were given a single i.v. dose of 75 μCi of [186Y]-CHX-A-DTPA-IgG at day 0 and a second dose of 75 μCi 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 ± 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 Human Monoclonal Antibody Radioimmunotherapy

The predicted protein has two F-spondin-like domains and a thrombospondin motif (Fig. 2). Human, rat, and mouse mindins are very similar, exhibiting ~85% identity at the protein level. A polyclonal rabbit antisera, RG-1/3C, raised against the peptide sequence shown in Fig. 2 reacted selectively with mindin/RG-1 in Western blots. By Western blot analysis, the protein is typically detected as a band that runs between the 38 and 47 kDa markers. This was seen in the prostate tumor cell line LNCaP which express mindin/RG-1 endogenously, and in PC-3 cells transfected with cDNA coding for the full-length protein (PC-3 cells themselves do not express RG-1/mindin message; data not shown). RG-1/3c also detected the protein in paraffin-embedded tissue sections (Fig. 3), and staining of sections could be efficiently blocked with 10-fold excess of competing peptide (Fig. 3F). The same staining pattern of Mindin/RG-1 protein in human prostate tissue was seen with the human biotinylated antibody 19G9 (Fig. 3F).

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.

**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<sub>d</sub> 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<sub>00</sub>-DTPA and radiolabeled with 111In, 90Y, or 86Y. The chelator CHX-A<sub>00</sub>-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 3 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 111In (13). The absence of antibody aggregates in the conjugate preparation was confirmed by size exclusion chromatography (data not shown). The CHX-A<sub>00</sub>-DTPA-19G9 antibody conjugate was radiolabeled with either 111In for biodistribution studies, 86Y for small animal PET imaging studies, or 90Y 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<sub>00</sub>-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 111In-labeled CHX-A<sup>+</sup>-DTPA-19G9 and an 111In-labeled isotype-matched human antibody that does not bind to mindin/RG-1 (CHX-A<sub>00</sub>-DTPA-IgG1), thus serving as a negative control. As shown in Table 2, [111In]-CHX-A<sub>00</sub>-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<sub>00</sub>-DTPA-IgG1. In order to visualize the tumor accumulation in LNCaP tumor–bearing mice, small animal PET studies were done using CHX-A<sub>00</sub>-DTPA-19G9 labeled with 86Y 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).

[86Y]-CHX-A<sub>00</sub>-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 μCi) of [86Y]-CHX-A<sub>00</sub>-DTPA-19G9 were injected i.v. into tumor (LNCaP)–bearing nude mice (n = 20; tumor volume, 95-425 mm<sup>3</sup>). An additional group was administered unlabeled antibody; a "no treatment" control group was also
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 proteins.
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 \(^{111}\text{In}, \quad ^{90}\text{Y}, \quad ^{90}\text{Y}, \) and \(^{177}\text{Lu}\). PET imaging studies with the positron emitter \(^{86}\text{Y}\) 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

### Table 1. Specific staining for mindin/RG-1 protein is seen in primary prostate tumors, locally recurrent tumors derived from androgen-insensitive patients and metastases to lymph node and bone

<table>
<thead>
<tr>
<th>Tissue source</th>
<th>Number of patient samples staining</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary untreated tumors</td>
<td>15 out of 18</td>
<td>moderate 2+ focal staining</td>
</tr>
<tr>
<td>Lymph node metastases</td>
<td>8 out of 10</td>
<td>moderate 2+ heterogeneous staining</td>
</tr>
<tr>
<td>Bone metastases</td>
<td>18 out of 23</td>
<td>moderate 2+/3+ heterogeneous staining</td>
</tr>
<tr>
<td>Locally recurrent tumors in androgen insensitive patients</td>
<td>22 out of 26</td>
<td>moderate 2+ heterogeneous staining</td>
</tr>
</tbody>
</table>

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.

### Table 2. Biodistribution of radiolabeled antibodies

<table>
<thead>
<tr>
<th></th>
<th>IgG(_1) (control)</th>
<th>19G9 (anti RG1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 3</td>
</tr>
<tr>
<td>Blood</td>
<td>12.8 ± 1.24</td>
<td>13.1 ± 0.72</td>
</tr>
<tr>
<td>Tumor</td>
<td>8.0 ± 1.55</td>
<td>7.1 ± 1.10</td>
</tr>
<tr>
<td>Tail</td>
<td>2.1 ± 0.23</td>
<td>2.0 ± 0.13</td>
</tr>
<tr>
<td>Skin</td>
<td>4.8 ± 0.33</td>
<td>5.3 ± 0.34</td>
</tr>
<tr>
<td>Liver</td>
<td>6.3 ± 0.91</td>
<td>6.1 ± 0.17</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.2 ± 0.41</td>
<td>1.3 ± 0.26</td>
</tr>
<tr>
<td>Intestines</td>
<td>2.5 ± 0.70</td>
<td>1.9 ± 0.26</td>
</tr>
<tr>
<td>Kidney</td>
<td>7.2 ± 0.38</td>
<td>6.9 ± 0.04</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2.1 ± 0.46</td>
<td>2.1 ± 0.10</td>
</tr>
<tr>
<td>Spleen</td>
<td>9.3 ± 2.64</td>
<td>8.2 ± 0.94</td>
</tr>
<tr>
<td>Heart</td>
<td>4.9 ± 0.50</td>
<td>4.1 ± 0.35</td>
</tr>
<tr>
<td>Lung</td>
<td>6.2 ± 0.60</td>
<td>7.4 ± 0.14</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.6 ± 0.08</td>
<td>1.6 ± 0.09</td>
</tr>
<tr>
<td>Bone (femur)</td>
<td>2.5 ± 0.46</td>
<td>2.4 ± 0.24</td>
</tr>
<tr>
<td>Brain</td>
<td>0.4 ± 0.06</td>
<td>0.5 ± 0.05</td>
</tr>
<tr>
<td>Testes</td>
<td>2.4 ± 0.17</td>
<td>2.7 ± 0.09</td>
</tr>
<tr>
<td>Seminal vesicles</td>
<td>1.2 ± 0.14</td>
<td>1.5 ± 0.02</td>
</tr>
<tr>
<td>Carcass</td>
<td>2.2 ± 0.13</td>
<td>2.3 ± 0.10</td>
</tr>
</tbody>
</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...
was explored in a preclinical study (26). 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',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 [90Y]-CHX-A00-DTPA-19G9 in a human prostate tumor (LNCaP) 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 had 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. [90Y]-CHX-A00-DTPA-19G9 was given at a single i.v. dose of 25, 50, 75, or 100 \(\mu\)Ci. Control groups included a group receiving unlabeled antibody alone (0 \(\mu\)Ci group) and a no-treatment group. Tumor growth inhibition of [90Y]-CHX-A00-DTPA-19G9 occurred at all doses of 90Y >25 \(\mu\)Ci, with the greatest effect seen at 75 and 100 \(\mu\)Ci (\(P < 0.05,\) 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 [90Y]-CHX-A00-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.

Figure 6. Antitumor effect of [90Y]-CHX-A00-DTPA-19G9 is prolonged after a second i.v. injection. LNCaP tumor cells were established s.c. in athymic nude mice. When tumors reached a volume of 50 to 400 mm\(^3\), animals were randomly distributed into treatment groups (n = 20) and given [90Y]-CHX-A00-DTPA-19G9 or [90Y]-CHX-A00-DTPA-IgG1, control antibody on day 0 as described previously. Animals in the control groups were euthanized once >30% of the animals had tumors >1,000 mm\(^3\). On day 49, animals previously treated with [90Y]-CHX-A00-DTPA-19G9 at 75 \(\mu\)Ci were randomized into two subgroups by tumor volume. One subgroup was given a second identical radioimmunotherapy dose (n = 10). A, progress of tumor growth as monitored by tumor volume is shown above. Points, means; bars, ± SE. Inhibition of tumor regrowth by a second radioimmunotherapy dose of 75 \(\mu\)Ci was observed for up to 90 days, when the study was terminated. B, body weight measurements and general clinical appearance were used to monitor toxicity. As shown, no toxicity as measured by body weight loss, clinical appearance, or death were observed in the course of the study. Points, means; bars, ± SE.
References


The authors gratefully acknowledge Dr. Martin Brechbhiel (NIH, Bethesda, MD) for providing the CHX-A\textsuperscript{2+} DTPA chelator; Drs. Richard Lafort, Ananya Raagana, and J. Yoo for coregistration imaging (Washington University School of Medicine, St. Louis, MO), Dr. Xiao-Yan Zhao for scientific discussions, Miriam Schroeder and Mithra Mahmoudi for technical assistance in performing immunohistochemistry studies, Rhonda Humm and Jean McRobbie for cell culture support (Bellerx Biosciences).

Acknowledgments


The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Identification of a Novel Prostate Tumor Target, Mindin/RG-1,
for Antibody-Based Radiotherapy of Prostate Cancer

Renate Parry, Doug Schneider, Debra Hudson, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/18/8397

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2005/11/09/65.18.8397.DC1

Cited articles
This article cites 29 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/18/8397.full.html#ref-list-1

Citing articles
This article has been cited by 10 HighWire-hosted articles. Access the articles at:
/content/65/18/8397.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.