Original Article

The Influence of Proteasome Inhibitor MG132, External Radiation, and Unlabeled Antibody on the Tumor Uptake and Biodistribution of [188]Re-Labeled Anti-E6 C1P5 Antibody in Cervical Cancer in Mice*

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BACKGROUND: Human papillomavirus (HPV) infection is considered a necessary step for the development of cervical cancer, and >95% of all cervical cancers have detectable HPV sequences. The authors of this report recently demonstrated the efficacy of radioimmunotherapy (RIT) targeting viral oncoprotein E6 in the treatment of experimental cervical cancer. They hypothesized that the pretreatment of tumor cells with various agents that cause cell death and/or elevation of E6 levels would increase the accumulation of radiolabeled antibodies to E6 in cervical tumors. **METHODS:** HPV type 16 (HPV-16)-positive CasKi cells were treated in vitro with up to 6 grays of external radiation, or with the proteasome inhibitor MG-132, or with unlabeled anti-E6 antibody C1P5; and cell death was assessed. The biodistribution of [188]Re-labeled C1P5 antibody was determined in both control and radiation MG-132-treated CasKi tumor-bearing nude mice. **RESULTS:** [188]Re-C1P5 antibody demonstrated tumor specificity, very low uptake, and fast clearance from the major organs. The amount of tumor uptake was enhanced by MG-132 but was unaffected by pretreatment with radiation. In addition, in vitro studies demonstrated an unanticipated effect of unlabeled antibody on the amount of cell death, a finding that was suggested by the authors' previous in vivo studies in a CasKi tumor model. **CONCLUSIONS:** The current results indicated that pretreatment of cervical tumors with the proteasome inhibitor MG-132 and with unlabeled antibody to E6 can serve as a means to generate nonviable cancer cells and to elevate the levels of target oncoproteins in the cells for increasing the accumulation of targeted radiolabeled antibodies in tumors. These results favor the further development of RIT for cervical cancers targeting viral antigens. *Cancer* 2010;116(4 suppl):1067–74. © 2010 American Cancer Society.

KEYWORDS: cervical cancer, viral antigens, radiolabeled antibodies, [188]Re, chemotherapy.

Greater than 95% of all cervical cancers are associated with and caused by the human papillomavirus (HPV),[1,2] a discovery that led to the receipt of the Nobel Prize by Dr. zur Hausen in 2008. Although HPV vaccination is approved by the US Food and Drug Administration, there are issues with implementation, access, and changes in cervical cancer screening as a result of vaccination. In addition, current vaccines are effective for preventing only HPV type 16 (HPV-16) and HPV-18 incident infection, and there is little benefit for women who already are infected with HPV-16 or HPV-18 or those who are infected with any other oncogenic HPV types. Increasing the potency of DNA vaccines still is among the

most important challenges for DNA vaccine development.[3] The impact of prophylactic vaccination on the incidence of the disease has yet to be determined, while millions of women remain at risk for cervical carcinoma worldwide. HPV strains use viral oncoproteins E6 and E7 to immortalize epithelial cells in culture and increase cellular transformation in concert with other oncoproteins.[4-6] The E6 and E7 oncoproteins are located intracellularly and bind to p53, promoting its rapid degradation through the ubiquitin-dependent pathway, whereas E7 oncoprotein binds to retinoblastoma (RB), thus causing ineffective cell growth regulation. By minimizing the effects of tumor suppressor genes p53 and RB, more random mutations can occur that potentially can lead to malignant transformation. Thus, E6 and E7 oncoproteins appear to be logical targets for targeted novel therapies for cervical cancer.

Radioimmunotherapy (RIT) is used experimentally for the treatment of various malignancies,[7] and 2 radiolabeled antibodies have been approved for treatment of recurrent or refractory non-Hodgkin lymphoma. In a previous report, we demonstrated the feasibility of targeting E6 and E7 oncoproteins in experimental cervical cancer by using radiolabeled antibodies as selective mediators of tumor destruction.[8] The distinctive features of this approach are: 1) the viral origin of target oncoproteins (as opposed to "self" human antigens used in prior RIT approaches), which obviates targeting host tissues; and 2) the intracellular and, in fact, intranuclear location of E6 and E7 oncoproteins. The targeting of intranuclear antigens is possible because degenerating and necrotic cells release their intranuclear contents and exhibit abnormal surface-membrane permeability, which allows reactivity of the antibody with intracellular antigen—characteristics that are not observed in normal cells. Thus, degenerating cells provide target material, because intracellular proteins dissipate from the damaged cell membrane and attract the radiolabeled antibody, which further mediates the destruction of viable tumor cells through long-range beta emission of a radionuclide such as ^{188}Re.

Clearly, the success of this strategy will depend on the quantity of target oncoproteins and their accessibility for binding antibody. Higher levels of target proteins and more nonviable cells that release such proteins would result in increased uptake of the radiolabeled antibody in the tumor. We investigated the use of external radiation, proteasome inhibitor MG-132, and pretreatment with unlabeled antibody to E6 as distinct means to generate nonviable cancer cells and to elevate the levels of target on-

coproteins in the cells for increasing the accumulation of radiolabeled antibodies in cervical cancer in nude mice.

MATERIALS AND METHODS

Cell Line, Antibodies, and Reagents

The CasSki cell line was obtained from American Type Culture Collection (Manassas, Va). Cells were grown in RPMI-1640 medium containing 10% fetal bovine serum (FBS) (Sigma Chemical Company, St. Louis, Mo) and 1% penicillin-streptomycin solution (penicillin, 10,000 U; streptomycin, 10 mg/mL; both from Sigma Chemical Company) at 37°C in a 5% CO_2 incubator. This cell line was derived from an HPV-16 -positive human cervical cancer that expresses both E6 and E7 oncogenic proteins. A murine antibody C1P5 (immunoglobulin G1 [IgG1]) to HPV-16 E6 and HPV18 E6 was procured from Abcam (Boston, Mass); human-mouse chimeric antibody tumor necrosis treatment 3 (ch-TNT3) IgG1 directed against a universal nuclear antigen was a gift from Dr. Alan Epstein (University of Southern California School of Medicine, Los Angeles, Calif). Proteasome inhibitor MG-132 was obtained from Calbiochem (San Diego, Calif), and BD Matrigel Basement Membrane Matrix was obtained from BD Biosciences (Rockville, Md).

Tumor Model

All animal studies were performed in accordance with the guidelines of the Institute for Animal Studies at the Albert Einstein College of Medicine. Thirty 6-week-old athymic Nu/Nu CD1 nude mice purchased from Charles River Laboratories were randomized into groups of 5 mice, and 10^7 cells were injected subcutaneous into the right flank of each mouse. For experimental development of a tumor model, the cells also were mixed before inoculation with combinations of RPMI medium, FBS, Matrigel, and MG-132. Mice in Group 1 were inoculated only with 10^7 cells; for other groups, mice were injected with 10^7 cells mixed with 3 μg/mL MG-132 (Group 2), 80% FBS (Group 3), 80% Matrigel (Group 4), 3 μg/mL MG-132 plus 80% FBS (Group 5), and 3 μg/mL MG-132 plus 80% Matrigel (Group 6). Tumor volume was calculated using length × width × height and divided by 2. Biodistribution experiments were performed when tumor volumes reached an average of 0.1 cm^3.

Antibody Radiolabeling

The beta-emitter ^{188}Re (half-life, 16.9 hours) was produced from beta decay of parent ^{188}W (half-life, 69 days) using a ^{188}W/^{188}Re generator (Oak Ridge National

Laboratory, Oak Ridge, Tenn). After [188]Re was eluted in the form of sodium perrhenate, the antibodies were labeled with [188]Re directly through binding of reduced [188]Re to the generated sulfhydryl groups on the antibodies as described previously.[8]

Biodistribution of [188]Re-Labeled C1P5 and Chimeric-TNT3 Monoclonal Antibodies in Nontumor-Bearing Mice

C1P5 monoclonal antibody (MoAb) to E6 and control MoAb ch-TNT3 were radiolabeled with [188]Re, as described above, with the specific activity of 1 μCi/μg and 20 μCi, respectively. [188]Re-C1P5 or [188]Re-ch-TNT3 was administered to nontumor-bearing nude mice as an intraperitoneal injection. At 6 hours and 24 hours, 8 mice (4 mice per antibody group) were killed, and their blood and major organs were collected at necropsy, weighed, and counted in a LKB 1282 Compugamma universal gamma counter (Wallac, Turku, Finland). The injected dose (ID) per gram of tissue expressed as a percentage (% ID/g) was calculated in counts per minute (CPM) according to the following equation: % ID/g = (CPM × 100)/(standard reference CPM × multiple factor × organ weight).

Determination of Effects of Radiation on CasKi Cells

For in vitro studies, CaSki cells were cultivated in a 24-well plate to a total volume of 1×10^5 cells/mL per well with RPMI medium supplemented with 10% FBS and 1% penicillin/streptomycin, and the cells were allowed to grow at 37°C in a 5% CO_2 incubator overnight. The medium in each well was removed, and the cells were washed with phosphate-buffered saline (PBS) 3 times. Five hundred microliters of fresh PBS were added, and irradiation at doses of 0 grays (Gy), 2 Gy, 4 Gy, and 6 Gy was performed on a Philips Orthovoltage MGC 40 Unit (Philips Healthcare, Andover, Mass) operated at 320 kVp, 5.0 mA at a 1.8 focal setting. After irradiation, PBS was removed, fresh medium was added, and the cells were placed back in 5% CO_2 incubator and grown at 37°C; then, cells were collected at different time points, and cell viability was determined by using the Trypan Blue dye exclusion method using a counting chamber (Neubauer hemocytomer) under the light microscope (Nikon Eclipse TS100; Nikon Corporation, Tokyo, Japan).

For in vivo studies, the tumor-bearing mice were immobilized using a cylindrical lead shield that allowed the tumors to be exposed but sparing the surrounding areas. The Philips Orthovoltage MGC 40 Unit was operated at the same settings that were used for the in vitro experiments to administer 6 Gy of radiation. Three days after irradiation, the mice were injected intraperitoneally with 20 μCi [188]Re-C1P5 MoAb, and biodistribution studies were performed 24 hours later, as described above.

Determination of Effects of MG-132 Proteasome Inhibitor on CasKi Cells

In our previous in vitro study, we used Western blot analysis to establish that the treatment of CasKi cells with concentrations of MG-132 from 2.5 μg/mL to 10 μg/mL for 3 hours to 6 hours significantly elevated the levels of target oncoprotein E6 in these cells.[8] In the current study, we investigated whether the same treatment also rendered the cells nonviable. For this purpose, the cells were grown in 24-well plates as described above; and MG-132 solution was then added at concentrations of 0 μg/mL, 1 μg/mL, 2 μg/mL, 5 μg/mL, 10 μg/mL, and 25 μg/mL. The cells were incubated under the same conditions for 3 hours or 6 hours, and cell viability was determined by using the Trypan Blue dye exclusion method.

For in vivo investigation of the influence of MG-132 on tumor uptake of the [188]Re-C1P5 MoAb, a group of 4 mice that carried CaSki tumors was injected intraperitoneally with 20 μg MG-132, and another group of 4 tumor-bearing mice was used as a control. Three hours later, all mice in both groups were injected intraperitoneally with 20 μCi [188]Re-C1P5 MoAb; and, 24 hours later, biodistribution studies were performed as described above.

Determination of Unlabeled C1P5 Antibody Effects on CasKi Cells

Previously, we observed a pronounced effect of unlabeled C1P5 antibody on slowing down the growth CasKi tumors in nude mice.[8] In the current study, we performed in vitro experiments to establish whether unlabeled C1P5 causes cell death. CasKi cells were grown in 24-well plates as described above and then were treated with concentrations of 0.013 μg/mL, 0.025 μg/mL, or 0.40 μg/mL C1P5 antibody or with the same concentrations of the control MoAb ch-TNT3. One 24-well plate was untreated, and another plate was treated with 5 μL lysis buffer at each time point according to the lactate dehydrogenase (LDH) kit guidelines (LDH Roche Cytotoxicity Detection Kit; Roche Applied Science Division of Hoffman-La Roche, Basel, Switzerland). Culture supernatant fluid was collected at 1 to 30 hours and frozen. The culture supernatant fluid was then thawed, and the cells were

removed by centrifugation at $\times 250g$ for 10 minutes. Then, 100 μL of the provided reaction mixture were added to the cell-free supernatant fluid and incubated while being protected from light for approximately 40 minutes at room temperature. Controls were prepared according to the LDH Roche Cytotoxicity Detection Kit instructions. The absorbance at 492 nm was then measured using an enzyme-linked immunoadsorbent assay reader (Roche Applied Science).

Statistical Analysis

Nonparametric Wilcoxon rank-sum tests were used to compare organ and tumor uptake in the biodistribution studies. The differences were considered statistically significant when P values were $<.05$.

RESULTS

Matrigel promoted the growth of CaSki tumors. We chose the HPV-16-positive CaSki cell line because it reliably expresses both E6 and E7 oncoproteins in vitro and in vivo.[8] However, in our experience, it took approximately 60 days from the inoculation of mice with CasKi cells for tumors to reach sizes from 3 mm to 5 mm in greatest dimension.[8] Consequently, we sought means to shorten this time by identifying experimental approaches that increased the tumorigenicity of the CasKi cells. The use of Matrigel, a solubilized tissue basement membrane matrix, significantly enhances the tumorigenicity of a wide variety of cancer cell lines in vivo (namely. breast, ovarian, endometrioid, lung, and prostate cancer cells; glioblastoma; and reports of enhancing growth of primary breast material from patients)[9]; however, to our knowledge, its effect on cervical carcinoma lines has not been studied to date. Therefore, we elected to test the effects of combinations of Matrigel as well as RPMI medium, FBS, and MG-132 on the tumorigenicity of CaSki cells.
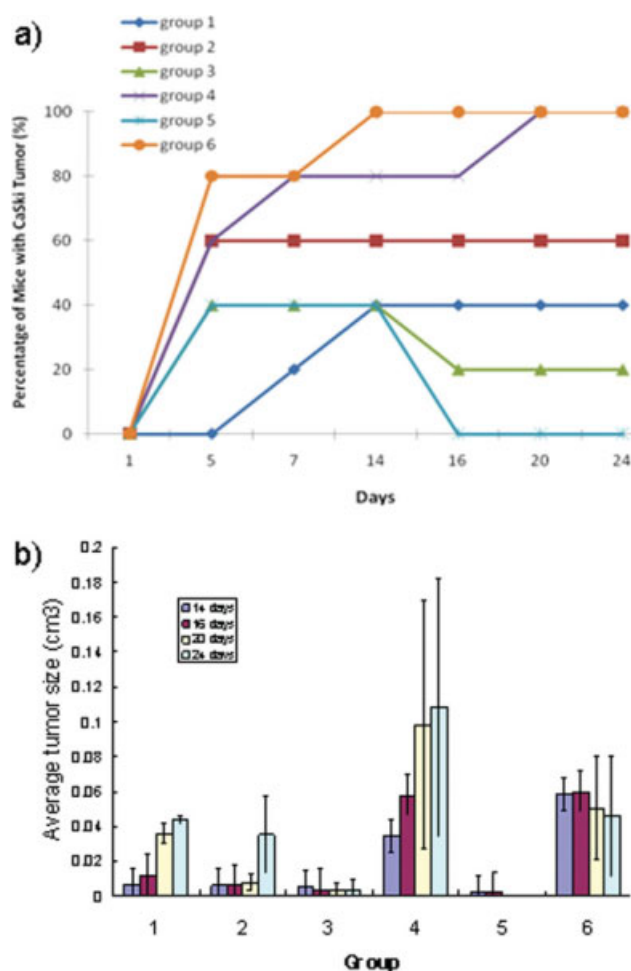
Matrigel promoted the formation of CaSki tumors (Fig. 1a). Groups 4 and 6 each had 100% tumor growth, with the effect of MG-132 demonstrating 100% growth at 14 days compared with Day 21 for Matrigel and medium alone. However, the overall tumor size was larger in Group 4, which received no MG-132 (Fig. 1b). FBS alone (Group 5) seemed to inhibit tumor growth over time, as evidenced by almost total regression of tumor during the observation period. It is possible that, in combination with MG-132, some inhibitory effects of FBS may be overcome; however, the overall tumor size (Fig. 1b) was not significantly enhanced.

Because the effects of Matrigel in promoting tumor growth were so pronounced, we performed a follow-up study of Matrigel inoculation alone by injecting it into the flanks of nude mice to determine whether Matrigel was able to recruit murine epithelial cells that would contribute to tumorigenesis. However, no resultant tumor was noted (results not shown), strongly suggesting that Matrigel alone does not cause tumor formation. In fact, it serves as a potent stimulator of tumor growth in the nude mouse cervical cancer model. Consequently, in all of these studies, we initiated CasKi tumors in nude mice using 10^7 CasKi cells mixed with Matrigel.

Antibodies to intracellular antigens cleared rapidly from tissues. Given our objective of investigating the in vitro effects of unlabeled MoAb to E6 C1P5 on CasKi tumors cells, we needed to ascertain that ch-TNT3 MoAb, intended as a control for this and future RIT studies, had similar C1P5 clearance patterns in normal organs. The MoAbs were radiolabeled with ^{188}Re, and their clearance was studied at 6 hours and at 24 hours. Both MoAbs demonstrated rapid clearance from the blood, liver, spleen, kidneys, stomach, and muscle, with ^{188}Re-C1P5 clearing faster at 6 hours, especially from the kidneys, and ^{188}Re-ch-TNT3 clearing faster at 24 hours (Fig. 2). We concluded that ch-TNT-3 had fast clearance because of low cross-reactivity with surface antigens and, thus, was a compatible control for intranuclear targeting.

External radiation did not increase the uptake of ^{188}Re-C1P5 in CaSki tumors, whereas MG-132 caused a significant increase in tumor uptake. The results of in vitro studies of cell viability after external radiation using doses from 0 Gy to 6 Gy demonstrated that the greatest number of cells with permeable membranes was produced by 6 Gy 72 hours postradiation (Fig. 3a). Therefore, we exposed CasKi tumors in mice to 6 Gy of radiation and administered ^{188}Re-C1P5 3 days later. No increase in the tumor uptake postradiation was observed; in fact, there was a trend ($P = .055$) toward a decrease in uptake (Fig. 3b).
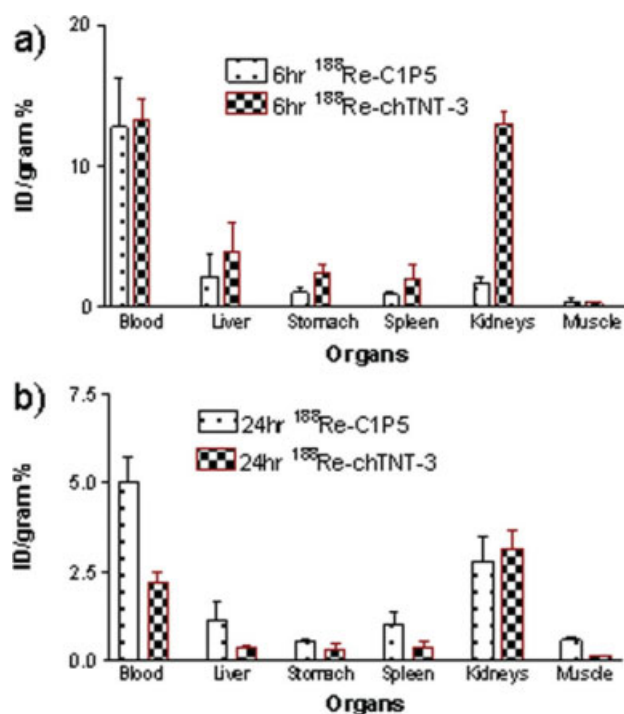
The treatment of CasKi cells in vitro with various concentrations of proteasome inhibitor MG-132 rendered approximately 8% of cells nonviable after 3 hours (Fig. 3c). Although 6-hour treatment produced more dead cells (Fig. 3c), the elevation of E6 expression in pretreated cells was the highest at 3 hours, and a significant decline in expression was observed at 6 hours.[8] On the basis of these data, a 3-hour time point was chosen, and CasKi tumor-bearing mice were injected intraperitoneally with 20 μg MG-132 (thus, the blood concentration

Figure 1. These charts illustrate the influence of various tumor growth promoters on the growth of CasKi tumors in nude mice. Matrigel promoted the growth of CasKi tumors more than other agents. Shown are (a) the percentage of mice with tumors and (b) the average tumor size (in cm³) in various groups. Mice in Group 1 were inoculated only with 10^7 CasKi cells; for other groups, the mice were injected with 10^7 cells mixed with 3 μg/mL MG-132 (Group 2), 80% fetal bovine serum (FBS) (Group 3), 80% Matrigel (Group 4), 3 μg/mL MG-132 plus 80% FBS (Group 5), and 3 μg/mL of MG-132 plus 80% Matrigel (Group 6).

Figure 2. Biodistribution of anti-E6 ^{188}Re-C1P5 monoclonal antibody (MoAb) and control ^{188}Re-human-mouse chimeric antibody (^{188}Re-ch-TNT-3) MoAbs in nontumor-bearing nude mice is illustrated (a) 6 hours after injection and (b) 24 hours after injection. ^{188}Re-C1P5 MoAb cleared faster at 6 hours than control ^{188}Re-ch-TNT3 and displayed low uptake in normal tissues.

would be 10 μg/mL, assuming a mouse blood volume of 2 mL) and received ^{188}Re-C1P5 3 hours later. Treatment with MG-132 resulted in a >2-fold increase in tumor uptake ($P = .03$) (Fig. 3d). It is noteworthy that no significant elevation in ^{188}Re-C1P5 MoAb uptake was observed in normal tissue (Fig. 3e).

Unlabeled C1P5 MoAb was cytotoxic to CasKi cells in vitro. In our previous report,[8] we noted inhibition of tumor growth in the nude mouse model when 30 μg (15 μg/mL blood) of unlabeled ("cold") E6 antibody

were administered. Here, we investigated the cytotoxic range of C1P5 concentrations by measuring LDH released into the supernatant fluid of treated cells as an indirect measure of cell death. The highest concentration of 0.4 μg/mL emulated the approximately 2% ID/g uptake of antibody in the tumor observed in our biodistribution experiments. Cytotoxicity was concentration dependent. There was no effect at C1P5 concentrations of 0.013 μg/mL or 0.025 μg/mL, whereas a C1P5 concentration of 0.4 μg/mL had pronounced effects on cell death (Fig. 4a). When the same concentrations of control MoAb ch-TNT-3 were applied, the significant lysis of cells was observed at a concentration of only 0.025 μg/mL, and 0.4 μg/mL caused a level of cellular death comparable to that caused by the C1P5 treatment (Fig. 4b).

DISCUSSION

Despite the major advances in early detection through cytologic screening with aggressive management of precancerous lesions and, now, with a preventative type-
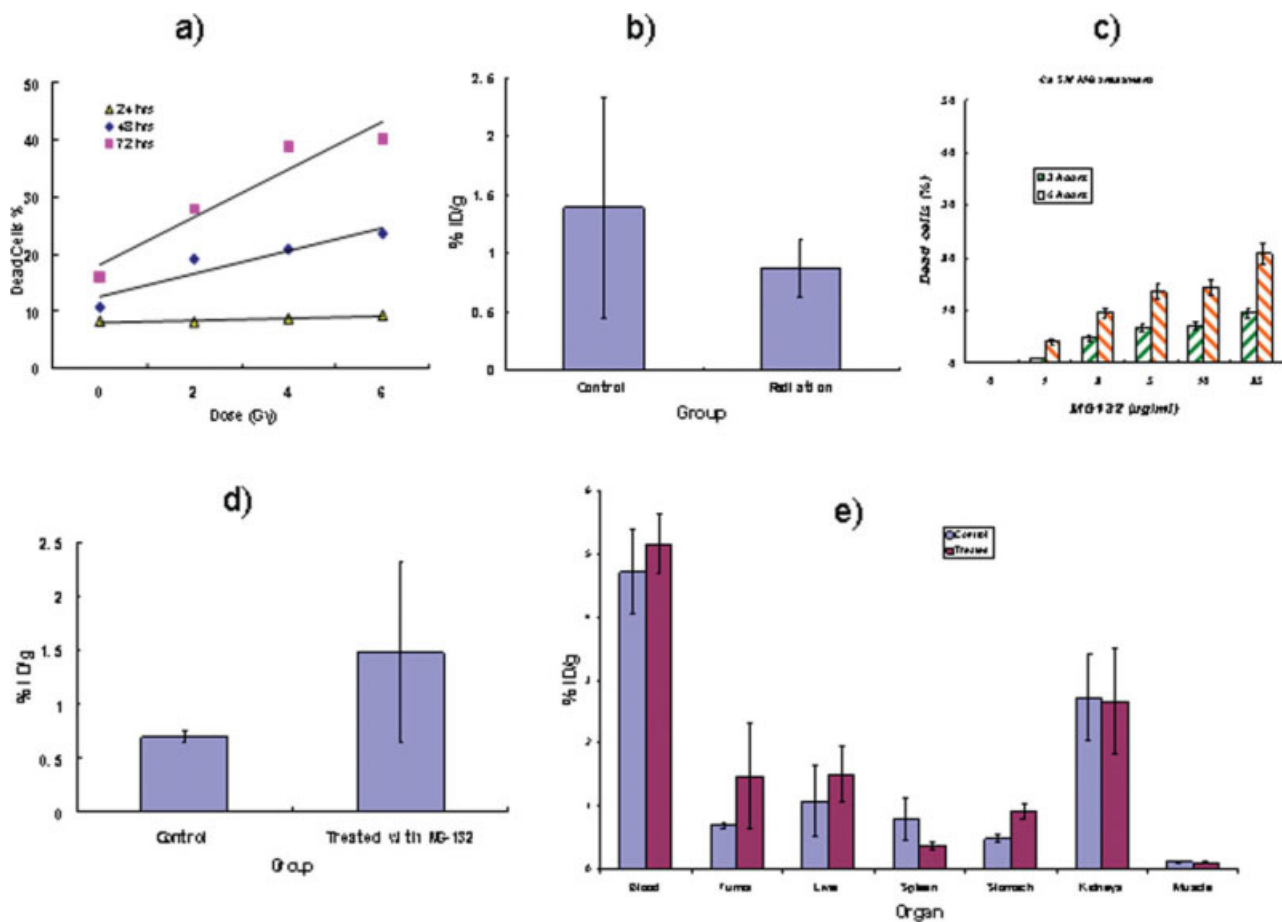
Figure 3. These graphics illustrate the influence of external radiation and proteasome inhibitor MG-132 on CasKi cell death in vitro and on tumor uptake and biodistribution of the [188]Re-C1P5 monoclonal antibody (MoAb) in vivo. (a) The numbers of dead cells after radiation exposure are shown. (b) This chart illustrates 24-hour uptake of [188]Re-C1P5 MoAb in CasKi tumors in mice that were exposed to 6 grays (Gy) of external radiation and were given [188]Re-C1P5 MoAb 3 days later. % ID/g indicates the percentage of injected dose per gram of tissue. (c) This chart illustrates the number of dead cells after MG-132 treatment. (d) This chart illustrates 24-hour uptake of [188]Re-C1P5 MoAb in CasKi tumors in mice that were treated with 20 μg of MG-132 radiation and were given [188]Re-C1P5 MoAb 3 hours later. (e) Organ uptake of [188]Re-C1P5 MoAb is illustrated in untreated and MG-132-treated CasKi tumor-bearing mice. Pretreatment of tumors with MG132 promoted the uptake of [188]Re-C1P5 MoAb, whereas external radiation did not.

specific HPV vaccine, cervical cancer remains the second leading cause of cancer death among women worldwide. For those women who have limited access to and poor medical care, cervical cancer is a significant cause of morbidity and mortality. Furthermore, the impact of surgery and the efficacy of radiation and chemotherapy for locally advanced cervical carcinoma are limited; and novel, well tolerated, therapeutic options urgently are needed.

MoAbs have clinical efficacy in the treatment of some advanced cancers.[7] Progress has been made in the application of MoAbs in the treatment of gynecologic malignancies, such as ovarian cancer. However, the success of these therapies has been limited.[10] Currently, to

our knowledge, there are no clinical trials of MoAb-directed treatment for patients with cervical cancer.

We recently reported that RIT targeting viral antigens could be used in the treatment of a broad range of virus-associated tumors, such as cervical cancer.[11,12] Many virus-associated cancer cells express viral antigens either on their surfaces or intracellularly. Intracellular viral antigens also are potential targets for RIT, because tumor cell turnover is likely to result in the release of these proteins into tumor interstitial spaces. It is important to emphasize that, when treating virally associated cancers by targeting viral antigen, not every cell in the tumor needs to express viral antigens for a therapeutic effect. Long-range
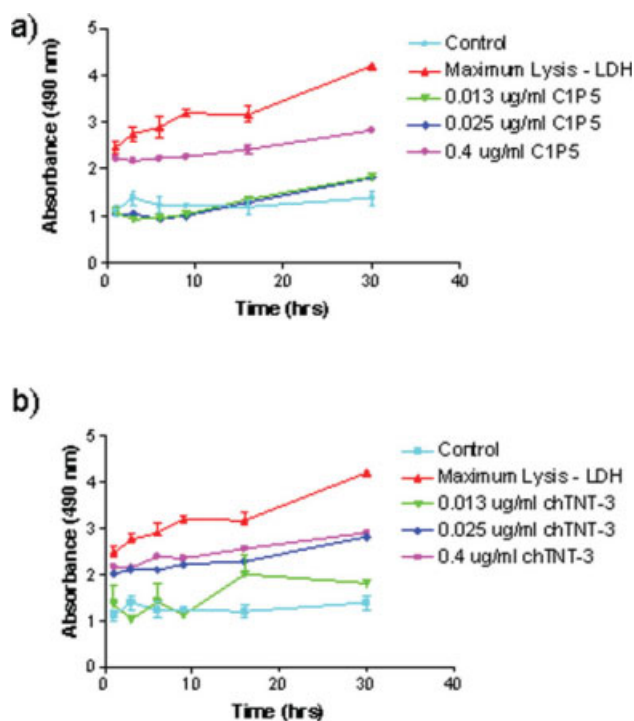
Figure 4. These charts illustrate the results from a lactate dehydrogenase (LDH) assay of CasKi cells that were treated with various concentrations of (a) anti-E6 C1P5 monoclonal antibody (MoAb) and (b) control human-mouse chimeric antibody (ch-TNT-3) MoAb. Control indicates that no antibody was added to the cells; Maximum Lysis, the cells were treated with lysis buffer. Treatment with unlabeled anti-E6 C1P5 MoAb caused cell death in CasKi cells.

emitters, such as [188]Re (emission range in tissue, 10 mm), emit radiation in a 360° sphere and, consequently, can kill viable tumor cells in the vicinity of the antigen location through the so-called "cross-fire" effect. [188]Re as a therapeutic radionuclide has other attractive features, such as a lack of accumulation in the bone marrow and rapid clearance through the kidneys.[13,14]

The current study demonstrates that antibodies targeting viral antigens, which also are intracellular, have very low cross-reactivity with normal tissues. The ability of such antibodies to bind to their respective targets retained by the nonviable cells would result in the increased residence times of the radiolabeled antibodies in the tumor. The clearance of [188]Re-C1P5 from organs in nude mice was more pronounced at 6 hours than in the control-treated [188]Re-ch-TNT3 mice. The latter binds to intracellular universal nuclear antigen[15] and clears significantly faster from the tissues compared with [188]Re-labeled IgGs to membrane-associated antigens.[16] Such low cross-reactivity

with normal tissues of MoAbs to viral antigens potentially can translate into low toxicity with RIT against viral targets.

To increase the amount and accessibility of target oncoproteins in the tumors, we investigated the pretreatment of tumors with external radiation, proteasome inhibitor MG-132, and unlabeled MoAb C1P5. Exposure to external radiation did not increase the uptake of [188]Re-C1P5 in tumors, possibly because of timing issues, vascular damage (which would prevent good circulation of antibodies in the tumor), or the inability of external radiation to damage nuclear membrane to release intracellular E6 oncoprotein. In contrast, pretreatment with proteasome inhibitor MG-132 increased the tumor uptake of [188]Re-C1P5 >2-fold. In this setting, the proteasome inhibitor MG-132 reduced the degradation of ubiquitin-conjugated proteins in mammalian cells without affecting ATPase or isopeptidase activities. MG-132 reportedly results in increased levels of E6 and E7 proteins in cervical cancer cells.[17,18] It is possible that pretreatment of tumor-bearing mice with a "classic" chemotherapeutic agent such as cisplatin also would result in killing some of the tumor cells and would lead to increased accessibility of target E6 protein. We recently demonstrated that pretreatment of experimental melanoma tumors with dacarbazine rendered some tumor cells nonviable, which made intracellular melanin pigment more accessible for the radiolabeled melanin-binding antibody.[19] In our previous study,[8] we demonstrated in vitro that MG132 treatment also increased expression of the E7 oncoprotein in other human cervical cancer cell lines, such as HeLa cells and SiHa cells, and we intend to perform experiments in mice bearing HeLa and SiHa tumors in the future.

It is noteworthy that the exposure of CasKi cells to unlabeled C1P5 MoAb at concentrations of 0.4 μg/mL, which would be achieved in the tumor at approximately 2% ID/g uptake (30 μg injected per mouse), caused significant cell death according to results of the LDH assay and may explain the pronounced effect of unlabeled C1P5 on CasKi tumors in mice in our previous study.[8] It also is possible that the cytotoxic effect of C1P5 on the cells contributed to the overall effect of RIT on CasKi tumors in mice[8] by increasing the number of nonviable cells in the tumor. The mechanism of this cytotoxicity is unknown and needs to be explored further.

In summary, the current study demonstrated that pretreatment of cervical cancers with the proteasome inhibitor MG-132 and with unlabeled antibody to E6

generated increased cell death of nonviable cancer cells. This resulted in elevated levels of target oncoproteins in the cells and increased the accumulation of radiolabeled antibodies in these cervical cancers in mice. These data will contribute to the further development of RIT for cervical cancers by targeting viral antigens and will add a potential therapeutic option for patients with this disease.

CONFLICT OF INTEREST DISCLOSURES

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