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Targeting the Virus with Radioimmunotherapy in Virus-Associated Cancers

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SUMMATION

"Virus-associated cancer" (VAC) refers to a cancer where viral infection results in the malignant transformation of the host's infected cells. Examples of viruses linked to cancers are the Epstein-Barr virus (EBV), which is associated with lymphomas, as well as nasopharyngeal and breast cancer; hepatitis B virus (HBV) and hepatitis C virus (HCV), which are both associated with hepatocellular carcinoma; and human papilloma viruses (HPVs), which are associated with cancer of the cervix. We have recently demonstrated that HIV-1-infected cells can be eliminated in vitro *and* in vivo *by targeting viral glycoproteins expressed on the surface of infected cells with radiolabeled viral protein-specific monoclonal antibodies and proposed that this approach can be applicable to the broad range of viral infectious diseases. In VAC, the tumor cells can exhibit viral antigens both internally or on their surfaces. As a result, viral antigens in tumors represent a potential antigenic target that is clearly different from normal tissues. In principle, these proteins could be targeted by radioimmunotherapy (RIT). In this paper, we describe the potential of this approach and review some of the issues involved in the development of this approach. RIT of VAC is fundamentally different from the previously described uses of RIT, which have targeted tumor-associated antigens that are "self" proteins.*

Key words: radioimmunotherapy, viral infection, virus-associated cancer

INTRODUCTION

"Virus-associated cancer" (VAC) refers to a cancer where viral infection results in the malignant transformation of the host's infected cells. Viruses linked to cancers in humans include the Epstein-Barr virus (EBV), which is associated with lymphomas, as well as nasopharyngeal and breast cancer; hepatitis B virus (HBV) and hepatitis C virus (HCV), which are associated with hepatocellular carcinoma; human papilloma viruses (HPVs) which are associated with cancer of the cervix; human T lymphotropic virus type 1 (HTLV-1) and type 2 (HTLV-2), which are associated with adult T-cell leukemia and with hairy-cell leukemia, respectively; and human her-

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pes virus 8 (HHV-8), which is associated with Kaposi sarcoma.[1–6]

In aggregate, VAC represents a large number of malignancies. For example, hepatocellular carcinoma is the fifth most common cancer and the third most frequent cause of cancer death worldwide, causing an estimated 550,000 deaths per year.[7] Chronic HBV infection affects over 400 million individuals worldwide and is the major risk for hepatocellular carcinoma.[8] Another example of the scope of the VAC problem is cervical cancer, which is caused by HPV. According to the World Health Organization (WHO), cervical cancer is the second most common cause of female cancer mortality in the world. WHO estimated the number of cervical cancer deaths to have been 250,000 in 2006. In the United States alone, cervical cancer has a major impact on women's health, with 10,500 new cases diagnosed in 2004 (American Cancer Society statistics). Hence, VAC represents a large group of heterogeneous tumors that have in common a viral etiology.

Conventional methods of VAC treatment include surgery, chemotherapy, and external beam radiation therapy (EBRT). Radioimmunotherapy (RIT), an alternative modality for the treatment of cancer that combines radiation and immunotherapy, started nearly three decades ago. In the United States in the mid-1980s, Order et al. carried out the first clinical trials of RIT for hepatoma.[9] RIT utilizes the specificity of the antigen-antibody interaction to deliver radionuclides producing cytotoxic doses of particulate radiation to tumor cells and provides a valuable alternative to chemotherapy and EBRT.[10,11] RIT has been successful in the treatment of lymphomas, with two Food and Drug Administration–approved radiolabeled monoclonal antibodies (mAbs), such as Zevalin® and Bexxar® (anti-CD20 mAbs labeled with 90-Y and 131-I, respectively). It seems likely that RIT will find a niche as a first-line treatment for follicular lymphoma.[12]

We have recently demonstrated that RIT has also a broad potential for the treatment of fungal and bacterial infections by targeting microbial antigens on the microbes with mAbs that are specific for these antigens.[13] Subsequently, we demonstrated that HIV-1-infected cells could also be eliminated *in vitro* and *in vivo* by targeting gp120 and gp41 viral glycoproteins expressed on the surface of infected cells with radiolabeled viral protein-specific mAbs[14] and proposed that this approach could be applicable to the broad range of viral infectious diseases and VAC.[15,*] In VAC, the tumor cells can exhibit viral antigens both internally or on their surfaces. As a result, viral antigens in tumors represent a potential antigenic target that is clearly different from normal tissues. In principle, these proteins could be targeted by RIT. In this paper, we describe the potential of this approach and review some of the issues involved in the development of this approach. This approach is fundamentally different from the previously described uses of RIT, which target tumor-associated antigens that are "self" proteins.

PRINCIPALS OF RIT FOR VAC

Several human neoplasias, carcinomas, and dysplasias and many more animal cancers are caused by virus infections (Table 1). The viruses that RIT is capable of targeting can be DNA or RNA viruses. In the VAC expression of virus-encoded genes, virus-encoded glycoproteins, and envelope proteins is a natural result of the infection. In VAC, viral antigens can be found in cell membranes or intracellularly and include a structural or a nonstructural protein of a virus, or a product of an oncogenic virus. Consequently, antibodies binding to viral antigens could be used for the delivery of cytotoxic radiation to the cancer. Although intracellular antigens are not ordinarily accessible to antibodies, cancers may include dead cells that have released their intracellular contents and, consequently, intracellular antigens are available for targeting.[16] Examples of intracellular viral antigens are the E6 and E7 oncoproteins of HPV16 and HPV18, which are responsible for 90% of cervical cancers. Similarly, in hepatocellular carcinoma caused by HBV, the virally encoded HBx protein is found in the nuclear matrix and attached to cytoplasmic membranes.

RIT can also be adapted to target nononcogenic viruses that preferentially replicate in tumor tissues. For example, enveloped RNA viruses, such as the vesicular stomatitis virus and the Newcastle disease virus, infect animals[17,18] and have an affinity for human cancer.[19] Hence, it may be possible to treat patients by infecting them with a virus that preferentially infects cancer and then follow up with RIT targeting antigens expressed

*U.S. Provisional Patent Application No. 60/876,052, filed on December 19, 2006.

Table 1. Examples of Human, Mammalian, and Avian Viruses Associated with Cancer

Virus	*Virus-associated cancer*
Epstein-Barr virus (EBV)	Lymphomas, as well as nasopharyngeal and breast cancer
Hepatitis B virus (HBV)	Hepatocellular carcinoma
Hepatitis C virus (HCV)	Hepatocellular carcinoma
Human papilloma virus (HPV)	Cancer of the cervix, head, and neck
Human T lymphotropic virus type 1 (HTLV-1)	Adult T-cell leukemia
Human T lymphotropic virus type 2 (HTLV-2)	Hairy-cell leukemia
Human herpes virus 8 (HHV-8)	Kaposi sarcoma
Bovine leukemia virus (BLV)	Bovine leukemia
Moloney murine sarcoma virus	Murine sarcoma
Avian myeloblastosis virus	Myeloid leukemia in chickens
Feline leukemia virus (FeLV)	Feline leukemia

by those viruses. Similarly, it may be possible to follow up gene therapies that utilize viral vectors, aiming RIT to target those therapeutic viruses in an attempt to eradicate residual cancer. Thus, the potential targets for RIT in VAC include the original antigenic virus, viruses with affinity for the cancer, and cancers treated with gene therapy delivered by viral vectors. Finally, it is apparent that it may be possible to develop therapies involving the delivery of viral antigens specifically to the cancer, followed by cytotoxic RIT in a two-step strategy, where the cancer is prepared for RIT.

The primary delivery molecules for the delivery of radioactivity are antibodies or their derivatives. In this regard, there is great variability of available reagents, and this includes mAbs of animal origin, humanized and chimeric whole mAbs, domain-deleted and single-chain Abs, hypervariable domain region peptides, Fv fragments and their multimeric forms, minibodies, and bispecific Abs.[20] A bispecific Ab[10] is an Ab molecule engineered to have two specificities, for example, a viral antigen and a cellular antigen. A bispecific Ab may be particularly useful if the cancer cell lacks a cognate virus receptor, as this provides the opportunity to improve the delivering of radioactivity to the cancer while reducing the dose to normal organs.

The choice of the radionuclides for VAC RIT should be determined by several factors, such as the location of the antigen (intracellular or cell surface), the size of the cancer to be treated, and its localization in the body. In the case of intracellular antigens, the carrier molecule tagged with a radionuclide will be able to bind to the antigen released from dying or nonviable tumor cells into the extracellular space, and viable tumor cells will be killed by "cross-fire" radiation. Beta (i.e., 188-Re, 90-Y, and 177-Lu), positron (i.e., 18-F and 76-Br), and mixed beta and positron (i.e., 64-Cu) emitters have a long emission range and thus are capable of a "cross-fire" effect. In this regard, we used 188-Re-labeled mAb and peptides that bind to melanin pigment (an intracellular antigen) released from the melanoma tumor cells as a result of a cellular turnover to treat melanoma in an experimental model.[21,22] For more accessible surface viral antigens or small tumors disseminated in the body, it may be possible to utilize alpha emitters (i.e., 212-Bi, 213-Bi, 223-Ra, 224-Ra, 225-Ra, 225-Ac, 212-Pb, 211-At, and 255-Fm), which have a short emission range in comparison to beta emitters. For the treatment of cancer cells in large tumors or those in difficult-to-access sites deep in the body, longer-lived isotopes, such as 90-Y (half-life, 2.7 days), 177-Lu (half-life, 6.7 days), or 131-I (half-life, 8 days), may be preferable.

ADVANTAGES AND POTENTIAL PROBLEMS OF RIT FOR VAC

"Traditional" RIT for cancer uses tumor-associated antigens that are "self" antigens in a patient's body, resulting in a significant uptake of the antibody in normal organs that may lead to toxicity. One challenge for immunotherapy is the identification of antigens that are specific to the cancer such that a maximum cytotoxic effect can be exerted while limiting damage to normal tissues. A monoclonal antibody (rituximab) directed against the B-cell surface antigen, CD20, is in-

creasingly being used as a therapy for B-cell lymphomas. However, CD20 is expressed on normal, mature B cells and hence is not a specific tumor target. In contrast to "traditional" RIT, virally transformed cancer cells are antigenically very different from host tissue, thus providing the potential for great specificity in targeting antigen-binding molecule interactions to the cancer cells. High specificity has the advantage of limiting the cross-reaction of radiolabeled binding molecules with host tissues, and this can be expected to result in less toxicity to normal organs than conventional RIT or chemotherapy. Another advantage of VAC RIT over traditional RIT is that it might find a use for the prevention of cancer in patients who are chronically infected with a virus by the treatment of precancerous virally infected cells before they transform into a type of cancer.

RIT treatment for cancer has several advantages over the related immunotoxin approach where a binding molecule, such as an antibody, is linked to a toxin molecule, such as ricin or diphtheria toxin. With RIT, a binding molecule used for radiation delivery does not need to be internalized to kill the cell, whereas an antibody toxin does. Furthermore, not every virus-infected cell in the body needs to be targeted by the binding molecule with RIT. I*n vivo,* there can be many cancer cells within a three-dimensional space where "cross-fire" radiation can be effective. By contrast, toxins only kill the cell in which they enter by virtue of conjugate binding. In contrast to toxins, radioisotopes do not elicit significant immune responses that can limit their subsequent use. RIT is also potentially less toxic, as the chemistry of linking different radioisotopes to antibodies has been well developed, and the exceptional stability of radiolabeled antibodies *in vitro* and *in vivo* has been confirmed.[11]

Based on the data accumulated in clinical RIT for cancer, the dose-limiting toxicity of RIT for VAC is likely to be bone marrow suppression. Important determinants of the extent and duration of myelosuppression include bone marrow reserve (based on prior cytotoxic therapy and the extent of disease involvement), total infection burden, and spleen size.[23,24] In addition, when using radioactive therapy in patients, there is always the concern for long-term effects, such as the subsequent development of neoplasms arising from radiation-induced mutations. However, this risk has proven to be very low following short-term exposure and is outweighed by the benefits of treating the cancer.

It is unlikely that particulate radiation utilized in RIT for VAC will cause the mutation of any virus whose genomic information is within a treated cancer cell. Viral replication already has an inherently high rate of mutation, and for RNA viruses, the mutation rate approaches the maximum rate compatible with continued viability.[25] Increased mutation rates, therefore, can reduce viral fitness, especially for RNA viruses. The virus genome is also physically small compared to that of a cancer cell nucleus, and therefore, is less likely to be damaged directly by the emitted radiation. Furthermore, ionizing radiation is a weak mutagen in comparison with chemical mutagens that are abundant in the environment.[26]

CONCLUSIONS

There is growing evidence that a large percentage of cancers are associated with viral infections in the host cells. RIT, for virus-associated cancers, targets viral antigens on the host cells and offers the possibility of exquisite specificity, so that it should result in decreased toxicity to the normal tissues. Another significant advantage of VAC RIT over traditional RIT is that it can be used for the prevention of cancer in patients who are chronically infected with a virus by the elimination of precancerous virally infected cells before they transform into a cancer. Nevertheless, considerable preclinical and clinical research is required to learn how to use RIT for VAC.

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About the Authors

Dr. Ekaterina Dadachova received her B.Sc. and Ph.D. degrees from Moscow State University in Russia. In 2000, Dr. Dadachova joined the Albert Einstein College of Medicine of Yeshiva University, where she is now an Associate Professor of Nuclear Medicine and Microbiology and Immunology. Her laboratory is currently working on targeted radionuclide-based therapies, in particular on the radioimmunotherapy of infectious diseases and metastatic melanoma. Using radioimmunotherapy for infectious diseases is a novel application of this technology because of an urgent need for new approaches to treat infectious diseases caused by the increasing prevalence of highly resistant microorganisms and by the occurrence of infections in immunosuppressed individuals in whom standard antimicrobial therapy is not effective. Another area of interest in Dr. Dadachova's laboratory is the treatment of breast cancer—the major cause of cancer death in women in the Western world—with receptor-binding small radioactive molecules, such as 18-fluorine-labeled glucose, that binds to glucose transporters, and 188-rhenium-perrhenate, which is a substrate for the sodium iodide symporter (NIS).

Dr. Xing-Guo Wang received his B.Sc. and M.Sc. degrees from Huazhong Normal University in China and his Ph.D. degree from Sheffield University in the United Kingdom. He serves as a professor in the Department of Life Sciences at Hubei University in China. His laboratory is interested in the biological role that microbial macromolecules, such as enzymes, lipoproteins, glycoproteins, and phospholipids, play in the interaction between microbes and eukaryotic hosts. In an attempt to identify microbial macromolecules as a possible therapy targets, his laboratory has focused on those proteins, that are expressed specifically in microbes, such as phosphotidyl-choline synthetase and bacterial laccases. In 2006, Dr. Wang joined Dr. Dadachova's research group at the Albert Einstein College of Medicine as a Visiting Scholar. He is currently working on targeting viral proteins in viral-associated cancer cells with radiolabeled antibodies to develop a radioimmunotherapy for these virus-associated cancers.

Dr. Arturo Casadevall is Chair of the Department of Microbiology and Immunology at the Albert Einstein College of Medicine of Yeshiva University. He also serves as a professor in the Departments of Medicine (Infectious Diseases) and Microbiology and Immunology and is the Leo and Julia Forchheimer Professor of Microbiology & Immunology. He received his BA from Queens College, CUNY and M.S., M.D., and Ph.D. degrees from New York University. His laboratory is interested in two fundamental questions: (1) How do microbes cause disease? and (2) How do hosts protect themselves against microbes? To address these questions, the laboratory has a multidisciplinary research program that spans areas of basic immunology and microbiology. A major focus of the laboratory is the fungus, Cryptococcus neoformans*, a ubiquitous environmental microbe that is a frequent cause of disease in immunocompromised individuals. In recent years, the laboratory has also worked with other microorganisms, including* Bacillus anthracis, Mycobacterium tuberculosis*, and* Histoplasma capsulatum.