A recombinant vaccinia virus containing the papilloma E2 protein promotes tumor regression by stimulating macrophage antibody-dependent cytotoxicity

Abstract Human papillomavirus infection is associated with cervical cancer. The E6 and E7 papillomavirus proteins are normally required for the maintenance of the malignant phenotype. Expression of these proteins in infected cells is negatively regulated by the binding of the papilloma E2 protein to the long terminal control region of the papilloma virus genome. The E2 protein can also promote cell arrest and apoptosis in HeLa cells. Therefore, it is clear that this protein has the potential of inhibiting the malignant phenotype. Because, anticancer vaccines based in vaccinia viruses have recently been shown to be an effective way to treat and to eradicate tumors, a recombinant vaccinia virus expressing the E2 gene of bovine papilloma virus (Modified Vaccinia Ankara, MVA E2) was created, to explore further the antitumor potential of the E2 protein. A series of rabbits, containing the VX2 transplantable papilloma carcinoma, were treated with MVA E2. An impressive tumor regression, up to a complete disappearance of tumor, was observed in most animals (80%). In contrast, very little or no regression was detected if the normal vaccinia virus was used. Lymphocytes isolated from MVA E2-treated rabbits did not show cytotoxic activity against tumor cells. However, in these animals a humoral immune response against tumor cells was observed. These antitumor antibodies were capable of activating macrophages to destroy tumor cells efficiently. These data indicate that injecting the MVA E2 recombinant vaccinia virus directly into the tumor results in a robust and long-lasting tumor regression. Data also suggest that antitumor antibodies are responsible, at least in part, for eliminating tumors by activating macrophage antibody-dependent cytotoxicity.

Key words Macrophages · Cytotoxicity · Tumor immunity · In vivo animal models

Introduction

Cervical carcinoma is the seventh most common cancer in the world. In 1996, an estimated 525 000 new cases were diagnosed worldwide, accounting for 5% of all new cancers [41]. Cervical cancer is the second most common cancer among women worldwide, accounting for 15% of all cancer deaths [39]. The highest incidence rates are observed in parts of Africa (35/100 000), Southeast Asia, and Latin America (26/100 000) [41]. Benign lesions, named papillomas, are small wart-like neoplasias that usually regress on their own. In some cases, however, there are lesions that undergo malignant transformation and develop into larger tumors.

More than 95% of all cervical carcinomas contain DNA of some human papillomavirus (HPV) [2, 6, 10, 31, 36, 37], with types 16 and 18 accounting for about 50% and 14% of all cases respectively [36, 38]. Papillomavirus (Papovaviridae family) are also found infecting a wide variety of vertebrates, including rabbits, camels, and pigs. These viruses have been described to produce tumors in these animals as well [30, 50].
Regular screening of abnormal cervical cytology (Pap smear) is an effective preventive strategy for cervical cancer [52]. When the disease is detected early and followed by an appropriate treatment, cancer patients present a good survival rate. However, despite the implementation of screening programs, many deaths are still recorded each year. Close to 2000 in the UK [5] and 12 000 in Mexico [1, 18]. When the disease is more advanced, traditional tumor therapy has unfortunately had only partial success in the case of cervical cancer. Recently, anticancer vaccines have proved a very promising alternative therapy for this type of cancer and have been shown to be the most effective way to treat and to eradicate virus-induced tumors [17, 33].

In the case of cell transformation by papillomavirus, the E6 and E7 viral proteins are normally required for the maintenance of the malignant phenotype [32]. These proteins achieve their effects by interacting with cellular anti-oncogenes, which normally have a negative regulatory role in cell proliferation. Two of the most important proteins that bind to these viral oncoproteins are the products of the tumor-suppressor gene p53 and the retinoblastoma gene (Rb) [7]. Expression of these viral oncoproteins in infected cells is negatively regulated by the binding of the E2 protein to the long terminal control region of the papilloma virus genome. However, the gene encoding E2 is frequently eliminated or inactivated when the virus genome becomes integrated into the cellular genome. This event results in high expression of the proteins E6 and E7, which can then exert their oncogenic properties, leading to tumor formation [7, 30, 54].

Because the E2 protein is responsible for regulating the papilloma oncogenes, there has been a lot of interest in its properties. Introduction of the E2 protein of papillomavirus into tumor has been found to promote cell growth arrest and stop cell proliferation [12, 13, 24]. A particular papillomavirus E2 protein can regulate a variety of different papillomaviruses, as shown for the bovine E2 protein, which can repress different HPV promoters [19, 22, 49]. The mechanism by which E2 inhibits tumor growth is complex. It not only down-regulates the E6 and E7 oncogenes but is also capable of inducing apoptosis of human cancer cells [12, 13, 24]. Moreover, the E2 protein seems to have a negative influence on cancer that goes beyond its direct effects on tumor cells. Immunization of animals with recombinant E2 proteins has been shown to induce tumor regression and decrease the number of new papilloma foci formed [44]. Clearly, the E2 protein has direct antitumor effects and also the potential of stimulating the immune system to recognize and eliminate malignant cells. These findings have led to a novel approach to cervical cancer therapy, namely the delivery of the E2 protein into HPV tumor cells. One of the most efficient ways to achieve this is to introduce the E2 gene into vaccinia virus vectors.

The vaccinia virus (Poxvirus family) has been used to vaccinate millions of people worldwide in the campaign to eradicate smallpox [16, 34, 35, 46]. Vaccinia vectors are attenuated viruses that direct the expression of foreign proteins in the cells they infect. These foreign proteins (antigens) can then be processed and transported to the cell surface as peptides coupled to MHC molecules, for presentation to the immune system [7]. In particular, a vaccinia recombinant vector, derived from the host-range-restricted and highly attenuated modified vaccinia ankara (MVA) strain of vaccinia virus [4, 7] has been used extensively for expression of various antigens [8, 23, 47, 48]. Inserting the gene of a protein into the vaccinia virus increases the expression of this protein in the infected cell and, in turn, the protein (antigen) stimulates the immune system more efficiently [3, 17, 40]. For all these reasons, we have used the MVA strain to construct a new recombinant virus carrying the E2 gene of bovine papillomavirus. This recombinant virus, named MVA E2, directed the expression of the E2 protein in infected cells, and was able to arrest human tumor growth in nude mice [51].

We now report that, in rabbits carrying the VX2 transplantable cottontail rabbit papillomavirus carcinoma [15, 20, 21], tumors stop growing and complete tumor regression may occur after treatment with the recombinant virus MVA E2. These rabbits were free of tumors for more than 1 year. They also presented specific antitumor antibodies that were capable of stimulating macrophages for efficient killing of tumor cells in vitro. In addition, passive transfer of these antibodies to new tumor-bearing rabbits resulted in tumor growth arrest. These data strongly suggest that the MVA E2 recombinant virus could be a promising anti-papilloma therapeutic agent.

**Materials and methods**

**Mice and rabbits**

Nude mice (*Mus musculus*), 8 weeks old, were purchased from Taconic Laboratory (New York, N.Y.). They were kept in “sterile” conditions in isolated cages. New Zealand white domestic rabbits were purchased from the University of Mexico (UNAM, Mexico City, Mexico) and were maintained in isolated cages in our animal house. All animals were kept according to good principles of laboratory animal care.

**Cells and viruses**

Monkey kidney (BS-C-1) and human carcinoma (HeLa) cells were maintained in a humidified air/5% CO₂ atmosphere at 37 °C. Chicken embryo fibroblast (CEF) cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (Gibco BRL, Gaithersburg, Md.), 20 µM glutamine, 50 units/ml penicillin and 50 µg/ml streptomycin. VX2 papilloma tumor cells were prepared as previously described [15, 20, 21] with minor modifications. Briefly, tumors isolated from mice were minced and washed in DMEM. Tumor fragments were then incubated with moderate stirring at 37 °C for 1 h in a 5-mg/ml collagenase solution in DMEM supplemented with 2.5% serum. Cell aggregates were then centrifuged (350g), washed with serum-free DMEM, and incubated with mild stirring for 30 min at 37 °C in a 2.5-mg/ml trypsin solution. Free cells were finally filtered through gauze, centrifuged and resuspended in serum-free DMEM. The