Strategies for Developing A Cervical Cancer Vaccine

Ricardo Rosales*

Introduction

Human papillomavirus (HPV) infection is associated with cervical cancer. Papillomaviruses can cause diseases, from warts and condylomas, to lesions which can progress to malignant neoplasias. Cervical cancer is a serious problem in developing countries because the disease is usually not detected early. In Mexico, several campaigns have been implemented since 1975 to detect the early stages of HPV infections in women. However, these campaigns have not been very successful, as indicated by the increasing number of patients with cervical cancer. In 1992 alone, 4,348 women died from cervical cancer, and this number has increased to a 5,000 average in recent years.

Many factors are associated with the development of cervical cancer: socioeconomic status, being over 45 years of age, multiple sexual partners, early sexual activity, first pregnancy at an early age, multiple pregnancies and smoking.

Usually human papillomavirus infects and replicates in epithilium cells, like the ones covering the vagina. These viruses produce proteins, including the E2 protein, that functions as a regulator for the production of two papillomavirus proteins, E6 and E7. After in-

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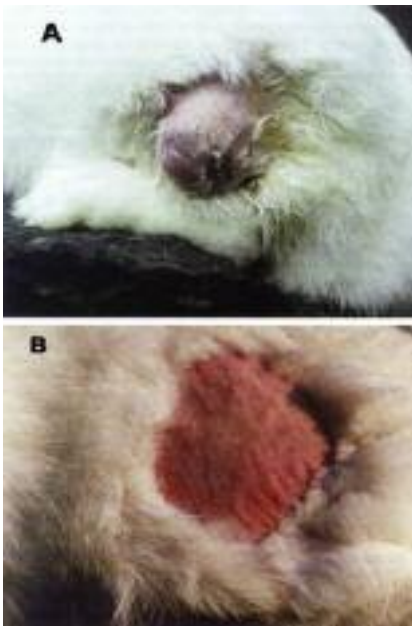
Ricardo Rosales working with mice.

* Researcher at the Institute for Biomedical Research (IIB), UNAM.



A) Tumor before treatment; B) Quadriceps' muscle area after treatment with MVA E2.

fection with the virus —for example, a year or two later— the E2 protein is destroyed, thus allowing E6 and E7 to reproduce in great quantities. Proteins E6 and E7 are responsible for changing a normal cell to a cancerous one. Thus, the E6 and E7 proteins are responsible for creating a cancerous tumor in a patient who has been infected with papillomavirus.

Because cervical cancer is a serious health problem, many approaches have been tried in an effort to develop a successful therapy. Surgery, radiation therapy and chemotherapy have, of course been used to reduce papillomas and cancerous tumors. Unfortunately, these methods only work efficiently during the first stages of tumor development. In later stages, it becomes very difficult to treat cervical tumors because of their size and the side effects that anti-cancer drugs may have.

Because cervical cancer has a close correlation to HPV infection, it is thought that inducing a protective stage against papillomaviruses would help in preventing cervical tumors.

Based on this idea, different strategies aimed at controlling papillomavirus have been tried.

Viral vectors (viruses used to manufacture vaccines), like, for example, the vaccinia virus, have been used for the design and manufacture of both therapeutic and preventive vaccines against cervical cancer. Using genetic engineering techniques, it has been possible to introduce genes into the vaccinia virus capable of producing proteins that have specific effects against other viruses or cancerous cells. Naturally, these proteins are recognized by the patient's immune system, which generates antibodies and specific cells capable of destroying viruses or tumor cells that contain the virus. The main strategy is making the vaccinia virus capable of producing proteins recognizable by the immune system and that generate a protective immunological response against viruses or cancer cells.

Another novel approach to controlling HPV infection and cervical cancer is based on the properties of the E2 protein of papillomavirus. This protein is capable of arresting cell growth and stopping cell proliferation by inducing apoptosis (or cell death) of human cancer cells. The E2 protein is also capable of inducing tumor regression and decreasing the number of new papilloma foci formed in animals immunized with the recombinant E2 protein (a protein that has been genetically modified to fulfill other purposes). Thus, the delivery of the E2 protein directly into HPV tumor cells should help arrest tumor growth. One of the most efficient ways to deliver a protein into cells is to place the E2 gene into vaccinia virus vectors (Poxvirus family).

Inserting an antigen in a vaccinia virus (poxvirus) increases the amount of these molecules in the infected cell, thus stimulating the immune system more efficiently. The purpose of using different antigens expressed in vaccinia virus is to try to enhance the immune response against these specific antigens. For these reasons it is thought that recombinant vaccinia viruses are excellent candidates for new types of vaccines as therapeutic agents. In addition, the vaccinia virus has been used to vaccinate millions of people worldwide in the campaign to eradicate smallpox.

An attenuated vaccinia virus known as Modified Vaccinia Ankara (MVA), has been developed and tested as a safe smallpox vaccine. This virus was found to be virulent for normal or immuno-suppressed animals and without side effects in 120,000 humans inoculated for the first time. One thing

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that makes the MVA very safe is that viral expression and recombinant mechanisms are impaired in this virus. This MVA vaccinia virus has other advantages. It is an excellent vector for expressing foreign genes, such as the *Escherichia coli* Lac Z or the page T7 polymerase in infected cells, and, moreover, MVA is capable of infecting most, if not all, the human cell lines tested up to now. Based on these characteristics, the use of vaccinia virus vectors is the most successful strategy for vaccine development today. Also, several other vaccines using the MVA virus against HIV, melanomas, measles, influenza, para-influenza, dengue virus, herpes virus and malaria have been successfully tested in animal models.

Ricardo Rosales at his microscope.

In our laboratory, we have tested the recombinant virus MVA E2 against HPV tumors. This recombinant MVA E2 virus contains the E2 gene of papillomavirus. When MVA E2 was injected directly into human HPV tumors in nude mice (mice without immune systems), tumor growth was arrested. In addition, when rabbits carrying the VX2 transplantable cottontail rabbit papillomavirus carcinoma were treated with the MVA E2 recombinant virus, their tumors stopped growing, and in 80 percent of the animals complete tumor regression was observed. These rabbits remained tumor-free for more than a year. They also presented specific antibodies that were capable of stimulating macrophages for efficiently killing tumor cells in vitro. In addition, passive transfer of these antibodies to new tumor-bearing rabbits resulted in tumor-growth arrest. This data strongly suggests that the MVA E2 recombinant virus is a promising anti-papilloma therapeutic agent. The findings described above work, and together with others, underscore the safety and effectiveness of using the MVA E2 recombinant vaccinia virus against cervical cancer, thus warranting further studies to investigate the therapeutic potential of MVA E2 in cervical cancer patients.

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A phase I clinical trial designed to test the safety of the MVA E2 recombinant virus was performed in 200 female patients having only papillomavirus infection but no lesions. The MVA E2 recombinant virus did not cause side effects in the patients, except in a few who experienced only a small increase in body temperature after application of the second dose.

No HPV DNA was detected in 50 percent of the patients treated with MVA E2 (using the hybrid capture method) after treatment. In contrast, in a parallel group only 20 percent of patients treated with cryosurgery did not have HPV DNA. A follow-up of MVA E2-treated-patients over a period of four years showed no recurrence of HPV infection in 35 percent of all patients as determined by colposcopy and Papanicolau tests. This showed that treatment with MVA E2 can eliminate the papillomavirus present in infected patients and also prevent new HPV infection. VM