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REVIEW

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Nuns, Warts, Viruses, and Cancer

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It has been known for more than 150 years that the risk of carcinoma of the uterine cervix correlates with the number of sexual partners. Laboratory and epidemiological evidence demonstrated that infection with certain human papillomavirus (HPV†) types initiates the vast majority of, if not all, cervical cancer, as well as a substantial fraction of other cancers, including other anogenital cancer and oropharyngeal cancer. Pap smear testing resulted in a dramatic reduction in the incidence of cervical cancer in the developed world, and HPV vaccination has the potential to eradicate HPV-associated cancer worldwide and represents a major public health breakthrough. The major current challenge is to ensure that HPV vaccines are widely administered.

The first clue was the nuns. In mid-19th century Verona, Dr. Domenico Rigoni-Stern observed that uterine cancer, while relatively common in women living in the city, was quite rare in the Catholic nuns who lived in the convents in the countryside [1]. What was the basis for this difference? Was it a miracle? Did the prayers of the good sisters ward off the disease? Further investigation revealed a more prosaic explanation: the risk of cervical cancer correlated with the number of sexual partners. Prostitutes had a relatively high risk of contracting the disease, married women in town had a moderate risk, and the celibate nuns (with the unfortunate, presumably rare, exceptions) were spared. This epidemiological profile strongly suggested that a sexually transmitted agent played an important role in the development of cervical cancer. Evidently, the good gentlemen of Verona had their guilty pleasures.

The search for the culprit lasted decades and had many false leads. Numerous agents infecting the genital region were falsely accused: syphilis, chlamydia, herpes simplex virus, and gonorrhea, a rogues' gallery of innocents.

The answer came from an unusual location: the plains of the Midwestern United States. In the early 20th century, trappers in the Midwest reported sightings of bizarre rabbits with horns. Some of these rabbits were captured and shipped from Kansas to the Rockefeller Institute in New York City for analysis. When the horns were ground up and a cell-free filtrate was inoculated into healthy rabbits, they, too, grew horns [2]. Microscopic examination revealed that these lesions were not horns at all, but rather highly keratinized warts or papillomas, benign tumors of epithelial cells. Electron microscopy showed that these lesions contained large numbers of virus particles. The first papillomavirus was discovered.

Unlike the warts on cottontail rabbits trapped in the wild, the warts produced by the virus on laboratory rabbits occasionally progressed to squamous cell carcinoma [3]. Thus, papillomavirus infections had carcinogenic potential. And what's true in rabbits is true in people. The French scientist Gerard Orth observed that papillomavirus infections can initiate cancer in humans suffering from epidermodysplasia verruciformis, a tongue-twister of a disease in which skin warts often progress to cancer, usually in areas exposed to sunlight, a well-known carcinogen [4].

Papillomaviruses were first implicated in cancer of the cervix in the early 1980s, with the report that the microscopic appearance of cervical cancer precursor lesions resembled cutaneous warts, known to be caused by human papillomavirus (HPV) infection [5]. Could HPV also cause cervical carcinoma? Harald zur Hausen in Heidelberg, Germany, took up the search. After unsuccessful attempts to demonstrate a role for herpes simplex virus type

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†Abbreviations: HPV, human papillomavirus; VLPs, virus-like particles

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2 in cervical cancer, zur Hausen turned his attention to papillomaviruses [6]. It was not a simple task because there are many different HPV types, which cause lesions at different anatomic sites. His initial studies showed that the HPV types that cause plantar warts on the feet and common warts on the hands were not present in cervical cancer. So zur Hausen then looked for viruses that infect the genital region. He started with HPV type 6, which caused external genital warts, or condyloma, cauliflower-shaped lesions on the genital skin [7]. Again, this virus was rarely present in cervical cancers. Rather than admit defeat again, zur Hausen used HPV6 DNA as a probe to detect the presence of viral DNA of distantly related, uncharacterized HPV types in cervical cancer tissue. Molecular cloning and sequence analysis of this DNA revealed new strains of HPV, types 16 and 18 [8,9]. Analysis of cervical cancers from many women revealed that fully 70 percent of them contained HPV16 or HPV18 DNA. zur Hausen and his team continued this boot-strap approach, isolating more and more divergent HPV genomes and capturing a larger and larger fraction of cervical cancers. Indeed, the prevalence of HPV DNA in cervical cancers approaches 100 percent, suggesting that the very rare HPV-negative cervical cancer was a diagnostic error [10].

These findings galvanized the biomedical community, which soon generated compelling evidence that HPV plays an essential role in the development of cervical cancer. Laboratory studies revealed that essentially all cervical cancers express HPV oncogenes that inactivate known cellular tumor suppressor proteins, such as p53, and that ongoing expression of these genes is required for continuous proliferation of the cancer cells. Studies in transgenic mice showed that these same genes can cause cancer in animals. Epidemiological studies demonstrated that infection with certain types of HPV conferred a greatly elevated risk of acquiring cervical cancer precursor lesions and cancer. These studies also showed that the cancers typically do not produce infectious HPV, many years usually pass between the time of infection and the development of cancer, and most HPV-infected women never get sick. For his seminal discoveries that launched this field, zur Hausen received a Nobel Prize in 2008.

The most convincing evidence that HPV initiates cervical cancer will come from the use of vaccines that prevent HPV infection. The papillomavirus particle is essentially a protein shell protecting the circular DNA genome. Remarkably, molecules of the major viral capsid protein can spontaneously assemble into non-infectious virus-like particles (VLPs), barren of DNA [11,12]. The external surface of VLPs so closely mimics the surface of the authentic virus that the immune system cannot distinguish it from the real thing. Therefore, when confronted with HPV VLPs, the body mounts a defensive barrage of antibodies and immune cells, which can interdict a subsequent natural infection. Thus, VLPs are very effective HPV vaccines. Clinical studies demonstrated that these vaccines prevented infection by the most common genital HPV types and greatly reduced the incidence of the precancerous cellular harbingers of cervical cancer [13]. Eventually, HPV vaccines are likely to reduce the incidence of cervical cancer itself, providing the final nail in the etiological coffin. However, because of the languid course of cervical carcinogenesis, women infected today will not get cancer for 20 years, and we will have to be patient before the ultimate success of HPV vaccines in preventing cervical cancer can be verified.

But HPV vaccination is not only a tool to demonstrate the importance of HPV in cervical carcinogenesis. It is also an important public health intervention that will save lives. Cervical cancer has been the target of other public health measures for many years, long before the link between cervical cancer and HPV was forged. This cancer develops over many years through a series of well-defined precursor lesions collectively called cervical dysplasia. George Papanicolaou discovered that dysplastic cells scraped from the surface of a diseased cervix could be identified by their morphology and staining characteristics [14]. This provided the basis for a screening test to identify women at higher risk of developing cancer and thus in need of curative medical intervention. In recognition of Dr. Papanicolaou, the test was named the Pap smear (a prescient hint that papillomaviruses initiated cervical cancer). Use of the Pap smear resulted in a dramatic decline in the incidence of cervical cancer in the developed world. However, cervical cancer remains the most common cause of cancer death in women in poorer areas of the world, where Pap smear testing has not been routinely implemented.

HPV vaccination has the potential to drive the rates of cervical cancer even lower, but in the United States, HPV vaccination rates are hovering at only around 40 percent in age-eligible girls and at an anemic 15 percent for boys [15]. It is important to vaccinate boys as well as girls, not only because boys can spread the infection to their sexual partners, but also because males themselves can fall victim to other HPV-associated cancers, including oropharyngeal cancer, which is rising rapidly in incidence [16]. There are hints that HPV vaccination is reducing the incidence of cervical dysplasia in Connecticut [15], but in more enlightened parts of the world where HPV vaccination has been enthusiastically adopted, the incidence of genital warts is falling not only in young women, who have been vaccinated for several years, but also in young men [17]. The males have not been vaccinated long enough to directly benefit from being vaccinated, but they are deriving benefit instead from the growing cohort of girls who are immune, a phenomenon known indelicately as herd immunity.

If HPV vaccines are deployed in the developing world and are as effective in practice are they are in trials, 10 percent of cancer deaths in women can be prevented [18]. This is an amazing accomplishment made possible by studies of a virus discovered in rabbits that made the long train ride from Kansas to the Big Apple.

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