

Harald zur Hausen's Experiments on Human Papillomavirus Causing Cervical Cancer (1976–1987)

From 1977 to 1987, Harald zur Hausen led a team of researchers across several institutions in Germany to investigate whether the human papillomavirus (HPV) caused cervical cancer. Zur Hausen's first experiment tested the hypothesis that HPV caused cervical cancer rather than herpes simplex virus type 2 (HSV-2), the then accepted cause. His second and third experiments detailed methods to identify two previously unidentified HPV strains, HPV 16 and HPV 18, in cervical cancer tumor samples. The experiments showed that HPV 16 and 18 DNA were present in cervical tumor samples. Zur Hausen concluded that HPV, not HSV-2, caused cervical cancer, which enabled researchers to develop preventions, such as the HPV vaccine.

From 1972 to 1977, zur Hausen worked at the Institute of Clinical Virology at the University of Erlangen-Nuremberg in Bavaria, Germany. There, he analyzed cervical samples from women for HSV-2 DNA, using the technique of nucleic acid hybridization. Zur Hausen introduced a single strand of HSV-2 DNA, composed of nucleic acids, the building blocks of DNA and RNA, into cervical tumor samples. If HSV-2 was already in the samples, the single strand would bind to its complementary half in the samples. Zur Hausen's nucleic acid hybridization repeatedly failed to find any HSV-2 DNA in the samples. Zur Hausen concluded that because HSV-2 DNA was absent from the samples, it did not cause cervical cancer. Zur Hausen then began reviewing medical reports about patients who had genital warts that later progressed to cervical cancer. Because HPV DNA had also been found in genital warts, zur Hausen hypothesized that HPV caused cervical cancer, rather than HSV-2.

HPV is a category of viruses in the family of papillomaviruses that infect humans through skin-to-skin or oral contact. The virus infects epithelial cells, which are cells that line the outer tissues and cavities of the body. There are many types of HPV, but only a small number cause cancer.

In February 1976, zur Hausen published "Condylomata Acuminata and Human Genital Cancer," stating his hypothesis that HPV caused cervical cancer. In the article, zur Hausen described his previous research experiments that failed to link HSV-2 to cervical cancer. He then proposed his theory that viruses can induce cancer, and if analyzed, the tumors would contain viral DNA. If the cancer tumors showed presence of viral DNA, then the virus would be an oncovirus, or a virus that causes cancer. He hypothesized that HPV-caused genital warts, condylomata acuminata, induced cervical cancer. Zur Hausen explained that previous experiments had demonstrated the presence of HPV in genital wart samples, distinguishing genital warts induced by HPV from warts caused by human wart viruses.

From 1980 to 1982, zur Hausen led a research team to determine whether HPV DNA was present in genital wart samples. The research team consisted of zur Hausen, Lutz Gissman, Matthias Dürst, Michael Boshart, Hans Ikenberg, and Dieter Wagner. Gissman directed the lab, and zur Hausen was the primary investigator of the research experiment, while the other researchers performed specific tasks. The genital warts and cervical cancer biopsies were from several hospitals in Germany, but the research was conducted primarily at the Institut für Virologie (Institute of Virology) at the Universität Freiburg (University of Freiburg) in Freiburg, Germany.

Zur Hausen and his research team studied HPV-caused genital wart samples and compared the DNA in the wart samples to the DNA in the cervical cancer tumors. Zur Hausen and his research team extracted HPV DNA from the tumor samples, and copied and amplified the HPV DNA in order to identify the strains. With the copied HPV DNA template matched to the HPV DNA in the tumor

samples, zur Hausen and his colleagues could identify the types of HPV that caused genital warts. Zur Hausen and his team identified two types of HPV that caused genital warts: HPV 6 and HPV 11.

Building on that research, in 1983, zur Hausen and his team investigated the presence of HPV 6 and 11 in cervical cancer biopsies from German, Brazilian, and Kenyan patients. Zur Hausen and his team extracted HPV DNA from tissue samples in the cervical cancer biopsies by inserting enzymes to isolate the HPV DNA from the rest of the samples. After the HPV DNA separated from the tissue samples, zur Hausen and his colleagues cloned the DNA multiple times to describe its underlying nucleotide sequence. They then compared the sequence to already established DNA sequences of HPV strains 6 and 11. While some of the HPV DNA sequence in the tumor sample matched to the HPV DNA strains 6 and 11, zur Hausen and his research team found inconsistencies. Certain fragments of DNA in the tumor samples did not match strains 6 or 11, leading them to designate the tumor sample HPV DNA sequence as belonging to an undescribed HPV strain, HPV 16.

Zur Hausen and his team re-analyzed the cervical cancer biopsies and looked for HPV 6, HPV 11, and HPV 16 DNA. Zur Hausen discovered HPV 16 DNA in over half of the cervical cancer biopsies in German patients. In comparison, he found HPV 16 in fewer than half of the cervical cancer biopsies in Brazilian and Kenyan patients. Zur Hausen and his team found little to no HPV 6 and 11 DNA in the cervical cancer tumor cells, leading zur Hausen to conclude that cervical cancer might be caused by a combination of HPV types, HPV type 16 as one of the main sources. Due to the difference in the prevalence of HPV 16 DNA in different patient demographics, zur Hausen determined that other HPV types may also cause cervical cancer.

A year later, zur Hausen and his team identified another new HPV strain in cervical cancer tumor cells. They continued their experiment from 1983 and screened for HPV 8, 9, 10, 11, and 16 HPV DNA in cervical cancer biopsies from German, Brazilian, and African patients. This time, however, when zur Hausen and his research team extracted HPV DNA out of a tumor sample from a Brazilian patient and described the HPV DNA, the isolated HPV DNA sequence did not match the any of the known HPV DNA strains. Zur Hausen identified the new strain as HPV 18. Zur Hausen and his team addressed the previous theory that cervical cancer might be caused by multiple types of HPV. They re-analyzed the cervical cancer biopsies and found HPV 18 DNA in nine out of thirty-six cervical cancer biopsies from African and Brazilian patients, and only two out of thirteen cervical cancer biopsies from German patients. The identification of HPV 18 further supported his previous theory that other types of HPV caused cervical cancer in addition to HPV 16, specifically HPV 18.

Once zur Hausen and his team identified that different HPV types could cause genital warts and cervical cancer, zur Hausen worked with another research team to further support his hypothesis that HPV 16 and 18 caused cervical cancer. Zur Hausen's collaborators included Ethel-Michele de Villiers, Achim Schneider, H. Miklaw, Uwe Papendick, Dieter Wagner, H. Wesch, and Jürgen Wahrendorf, who conducted the research at several institutions in Europe.

In 1987, zur Hausen analyzed HPV 6, 11, 16, and 18 infections in 9,295 Pap smears from female patients in three gynecological hospitals in three different regions in southern Germany. Zur Hausen and his team found that 8,755 Pap smear samples were normal, presenting no abnormal cells. They found that 196 Pap smear samples demonstrated koilocytotic HPV infection, or halo-like cells in the layers of the epithelial tissues. One hundred sixty-two Pap smears were abnormal and were identified as mild cervical cancer, 120 Pap smear samples were identified as a higher stage of cervical cancer, and sixty-two Pap smear samples showed an invasive level of cervical cancer.

Zur Hausen and his team determined that the of the 8,755 normal Pap smear samples, a small percentage tested positive for HPV DNA, demonstrating that other HPV types are present in cervical cells and do not cause cancer. Zur Hausen and his team also found a strong prevalence of HPV 16 and 18 DNA and little evidence of HPV 6 and 11 DNA in the Pap smears where mild or moderate stages of cervical cancer was present. About eighty percent of the abnormal Pap smear samples that demonstrated cervical cancer showed presence of HPV DNA, confirming the theory that HPV caused cervical cancer. Zur Hausen and his research team concluded that HPV 6 and 11 caused genital warts, while HPV 16 and 18 caused cervical cancer.

Once zur Hausen identified HPV types 16 and 18 and found prevalence of HPV 16 and 18 DNA in

cervical cancer tumors, other scientists confirmed that HPV caused cervical cancer and determined that HSV-2 was a co-factor to cervical cancer. The results of zur Hausen's experiments helped identify many more HPV types and were used as a template to develop the HPV vaccine, a preventative measure to help decrease the risk of cervical cancer, a common reproductive cancer in women. The vaccine was approved in 2006 for use in the US. In 2008, zur Hausen received the Nobel Prize in Physiology or Medicine for discovering HPV caused cervical cancer.

Sources

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