

Finding the viral link: the story of Harald zur Hausen

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German virologist Harald zur Hausen was convinced by the early 1970s that the skin wart virus, human papilloma, was implicated in cervical cancer. Thirty years later we stand on the threshold of a vaccine to prevent this major killer of women. Looking back, it could have happened earlier...

Two pharmaceutical giants recently announced sensational results from clinical trials of vaccines that could prevent at least 70% of the 470,000 cases of cervical cancer globally each year. Cervical cancer kills 230,000 women a year, eight out of ten of them in developing countries, where it is the most common cancer in women.

In November 2004, the GlaxoSmithKline HPV vaccine study group delivered the results of a three-year study of a vaccine to protect against human papilloma virus (HPV). They reported in *The Lancet* 100% efficacy against persistent HPV 16 and 18 infection, the two types most closely associated with cervical cancer. The team called for long-term follow-up to confirm that this would prevent cervical cancer, but concluded “our data provide compelling evidence that the HPV 16-18 vaccine is highly efficacious” and “appeared to be safe and well tolerated”.

Last month, at the 22nd International Papillomavirus Conference in Vancouver, a team from Merck reported that their vaccine had

shown 90% protection against HPV 6, 11, 16 and 18. Alex Ferenczy, from McGill University, one of those involved in the Merck trial, said: “These are very exciting times for all of us in the field of cervical cancer prevention.” His colleague Philip Davies, head of the European Cervical Cancer Association, went further. “We have the means to virtually eliminate cervical cancer.”

Amongst those present in Vancouver was Harald zur Hausen, from Heidelberg, who can see the development of these vaccines as a vindication of his lifetime’s work, and could be forgiven for thinking that this could have happened some years earlier. It was zur Hausen who first showed that the papilloma virus is the most significant cause of cervical cancer, and he stuck to his beliefs through years of confusion when the role of viruses was largely discounted.

A LIFE’S WORK

When Harald zur Hausen qualified as a doctor in 1960, the role of viruses in human cancers was unknown.

The first tumour-inducing virus had been dis-



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covered in 1911. Peyton Rous at the Rockefeller Institute for Medical Research in the US had isolated, from chickens, Rous sarcoma (retro)virus (RSV), which caused tumours in animals. Rous went on to pioneer research into the rabbit papilloma virus and its interactions with chemical carcinogens in the 1930s, and received a Nobel Prize for his work in 1966. In the early 1960s Ludwig Gross in New York demonstrated that retroviruses caused tumours in mice and rats.

zur Hausen's interest in infectious diseases and microbiology began when he was a student doctor in the 1950s. “Maybe it is the difficulty of the problems that fascinated me. I was certainly interested initially in the infectious causes of diseases, rather than cancer.” In 1961, his first

job was at the Institute of Microbiology in Düsseldorf, where he spent three years trying to induce a vaccinia (cowpox) virus to produce chromosomal breaks in mouse cells.

“This virus and many others did something to the chromosomes, but nothing very characteristic. I didn't get very much help because nobody else was interested in that question in that place. They just left me to it. At a much too early stage I was very independent and without sufficient background in the field.”

He took courses in cytogenetics and molecular biology and taught himself to do lab work. “I got increasingly frustrated with my situation,” he recalls. “After much too long, I decided to look for a position somewhere else.”

The audience listened to zur Hausen in stony silence, and dismissed his (now vindicated) results

Then came one of those random mutations that seem to influence most successful careers. The School of Medicine at Pennsylvania University wrote to the Institute asking for a young German Fellow to come and work in the US. The Director threw the letter away, but later mentioned it to the young colleague with the interest in vaccines. zur Hausen did not hesitate. "I fished the letter out of the trash can, and went to Philadelphia."

There, renowned virologists Werner Henle and his wife Gertrude Henle were studying the Epstein-Barr virus, which had been observed the previous year in the UK in cultured Burkitt's lymphoma cells, using an electronmicroscope. The virus induced changes in human chromosomes and zur Hausen found the work interesting and intriguing. "The Henles very gently showed me what I did not know and I gained a lot of technical expertise and experience."

He used nucleic acid hybridisation to analyse DNA, and a fluorescent test developed by the Henles to detect the virus in a very few cells.

The Philadelphia experience inspired him, but zur Hausen disputed the view, held by the Henles, that cultured Burkitt's lymphoma cells maintain a persistent infection, in which a few infected cells transmit the virus to a small number of others.

zur Hausen's view was influenced by lyso-genic bacteria, where the DNA of a bacteriophage persists in all bacterial cells and may become activated to produce virus in an occasional cell. He speculated that Epstein-Barr virus may persist in all Burkitt's lymphoma cells but become spontaneously activated only in a very limited number of cells.

After three years, zur Hausen was offered his own laboratory at the Institute of Virology in Würzburg, and he returned to Germany, determined to put his theory to the test. After a long and difficult struggle he was eventually able to

show that "non-virus-producing" Burkitt's lymphoma cells contained the Epstein-Barr DNA. "We showed for the first time that viruses can persist in human tumour cells as genomes, and probably modify via the genomes these cells into tumour growth." It was not entirely unexpected to find signs of the virus in lymphatic cells, since Epstein-Barr virus can cause mononucleosis, a disease that involves the lymph nodes. But, zur Hausen and his colleagues were also able to demonstrate the virus in cells taken from a nasopharyngeal tumour, an epithelial carcinoma.

STEADY APPROACH

By the end of the 1960s, zur Hausen had a growing reputation. However, his careful, rational approach was not always heard in an age with huge appetite for social and scientific advances and in a field which seemed to induce wild optimism or profound scepticism.

The youngest of four children, as a young child near Essen in the final years of the war, Harald had witnessed the destruction of this industrial part of Germany in daily bombing raids. For young people in post-war Germany life was a serious business, and the zur Hausen children focused on their studies. Later, the hedonism of the late 1960s passed him by. "I was in places in Germany that were very quiet or in Philadelphia. I was not so much part of the 1960s. I was never a hippy," he recalls.

Perhaps this helped him to keep his feet on the ground.

At Würzburg he became increasingly sceptical about claims that cervical cancer, which was clearly sexually transmitted, was caused by the herpes simplex virus.

In 1972, at the age of 36, he was appointed Professor of Virology at the University of Erlangen-Nuremberg in Bavaria, and set up a programme to examine other candidates, including the papilloma virus (HPV), responsible for

VIRAL CANCERS

Viruses are strongly associated with about 15% of the global burden of cancer.

- Human papilloma virus with cervical cancer
- Hepatitis B&C with liver cancer
- Epstein-Barr virus with Burkitt's lymphoma and nasopharyngeal cancer and lymphomas
- The human T-cell lymphotropic virus (HTLV1) with adult T-cell leukaemia
- Human herpes virus type 8 (HHV-8) with Kaposi's sarcoma

A viral aetiology is also suspected for other lymphatic cancers, leukaemias and some brain tumours. Other tumours are linked to bacterial and parasitic infection. *Helicobacter pylori* is a major cause of gastric cancer, and parasitic liver flukes are responsible for a significant proportion of liver cancers in South East Asia. Globally, infections cause about 20% of cancers, but they are not evenly spread. In sub-Saharan Africa and parts of Southeast Asia about 40% of cancers are acquired through infections. In Western Europe and the US it is more like 10%.

skin warts. This cannot be grown in tissue cultures and is difficult to isolate from clinical specimens such as genital warts, where it exists in very low particle concentrations.

They were able to extract papilloma DNA from virus particles in the plantar wart (verruca). To zur Hausen's disappointment, these did not react to the genital warts, implying that the viruses must be different. Nor did they react to other skin warts which contained the virus. He was discovering that papilloma is not a single virus but many. This heterogeneity was also demonstrated in Paris. Today, we know of 106 different genotypes of papilloma virus and there are probably more to come.

At the time, much of the work on viral causes of cancer was being conducted in the US, under the Virus Cancer Program that had been set up in 1964 by the National Cancer Institute with a budget of US\$50–60 million a year. This work focused on retroviruses, following the discovery of the feline leukaemia virus in cats, the bovine leukaemia virus in cattle and the ability of retroviruses to induce cancers in rats and primates.

This work eventually led to the discovery of oncogenes, but DNA virus research was neglected and poorly done.

In 1973 the US National Cancer Advisory Board set up an investigation into the Virus Cancer Program, which criticised its lack of attention to DNA viruses, the fact that grants

went to a limited number of laboratories, and the way that researchers could vote money to each other. The report was designed to refocus the programme, but the message that went out to the public was that most research into viruses and cancer was a waste of money.

LOW POINT

In 1974, zur Hausen went to an international conference in Florida to present results showing that herpes simplex was not present in cervical cancer. Shortly before he was due to speak, a researcher from Chicago announced that he had isolated 40% of the herpes simplex genome in one cervical cancer specimen. The audience listened to zur Hausen in stony silence, and dismissed his (now vindicated) results as lacking sensitivity. It was the low point of his professional life.

In 1977 zur Hausen took the Chair at the Institute of Virology at Freiburg. His team was able to extract and type virus material from a genital wart. Disappointingly, this type (HPV 6) was not present in cervical cancer cells. Soon afterwards the team isolated HPV 11, and found distantly related sequences in a cervical cancer biopsy. Next, Mathias Dürst, then a student at the Institute, succeeded in cloning a new type, HPV 16, from a cervical cancer biopsy. They were immediately able to show that this was present in about half of cervical cancer biopsies. The Institute then isolated HPV 18,

In 1984, pharmaceutical companies turned down zur Hausen's request to work on an HPV vaccine

responsible for another 17%–20% of cervical cancers.

In 1983 zur Hausen became director of the German Cancer Research Centre (Krebsforschungszentrum) at Heidelberg, and he gave over much of his time to refocusing the way that the research was done, introducing peer review, and breaking down barriers between separate research institutes. He encouraged researchers to rely less on mouse models and to work more closely with clinicians. He launched clinical co-operation units with University hospitals and in the last two years of his directorship, established the foundation for a comprehensive cancer centre with the University of Heidelberg.

In 1984, more than 20 years ago, zur Hausen approached pharmaceutical companies to work on developing a vaccine against HPV, which he was now convinced caused the vast majority of cervical cancers. "The viruses had quite a simple structure and it should have been possible to produce something. But the companies I approached did not believe that this would be profitable and said there were more urgent problems to be solved."

In the mid-1980s the polymerase chain reaction (PCR) 'democratised' genetic research, putting a quick and easy method for copying DNA fragments within reach of biologists with little training in molecular biology or how to prevent cross-contamination of results. Laboratories all over the world began reporting HPV 16 in all kinds of tissue.

LOST OPPORTUNITY

These errors were enough to close the window of opportunity to hunt for a vaccine. zur Hausen says: "People became sceptical about the role of papilloma viruses in cancers. Pharmaceutical companies were not interested in the story any more, because this period created such confusion. I got a bit frustrated in this period and could

not hide it. Cervical cancer is one of the major cancers worldwide, and it kills relatively young women. If our original conviction that this virus must be causative had been carried through, we would have made an earlier start on a vaccine."

By 1991, a number of epidemiological studies confirmed that the papilloma virus was indeed the causative agent for cervical cancer.

In March 2003, now highly decorated and honoured, zur Hausen retired as director of the Krebsforschungszentrum. He continues to work at the centre where his wife Ethel-Michele de Villiers, a Professor of Virology, keeps a depository of all 106 known papilloma types. This allows him what he calls "the privilege of friendly interference".

The Centre is still devoted to the aetiology of tumours, searching for viruses in leukaemia and lymphoma, especially the 80% of cases of Hodgkin's disease that do not contain Epstein-Barr virus. zur Hausen is researching lymphomas and leukaemia in children, for which a clean home and protected environment are risk factors, while poor hygiene and day care are protective. He believes that the intermittent infections that children acquire in less protected environments disrupt the build-up of the persistent infection which can lead to leukaemia.

He is also interested in how the normal protective mechanisms that the body has against the creation of 'immortalised' cancer cells are turned off one by one. The immune system, a system to block viral oncogenes and the ability of cytokines to render tumour cells harmless are all normally highly efficient, and they have to be switched off. It takes a long time for this to happen, but tumours have plenty of time. Infection with papilloma virus often occurs between the ages of 15 and 22. Cervical cancer is most common between the ages of 40 to 45. This 20-year period is sufficient for the genetic legacy of the virus to disarm cell growth regulators one by one.

Clearly the virus is not the whole story. Because, while men too have the virus, rates of penile cancer are barely 5% of the rates of cervical cancer. It is possible that oestrogen stimulates virus-producing cells and the 'immortalisation' of cancer cells.

UNANSWERED QUESTIONS

Other factors such as smoking make cancers more likely, but zur Hausen believes that the papilloma virus will do it alone, given time. "People say that the papilloma virus is a necessary but not a sufficient factor. We here are deeply convinced that this statement is wrong; that the virus is necessary and in quite a number of instances is also sufficient. Modifications in the host cell genome can of course occur due to chemical or physical carcinogens, but they also occur due to the mutational activity of the viral oncoproteins themselves. Their long-term expression leads to that accumulation of mutations which may lead to tumours.

"Provided you give it time and provided it is not cleared by the immune system, then the risk is high that a woman develops cancer. We see today that the previous strict separation between chemical, physical, and biological carcinogens is nonsense. There is a very close inter-relationship between these factors."

A few scientists still report cervical cancer without papilloma virus. zur Hausen will believe it when he sees it. "I frequently ask colleagues to provide us with tumour samples which are in their opinion negative and we have never got any."

Known HPV types account for about 90% of cervical cancers, and it is possible that others are each responsible for a small part of the total. Some 'low-risk' HPV types, such as 6 and 11, very rarely can also cause other cancers.

zur Hausen believes there will eventually be one vaccine covering almost all the high-risk

papilloma virus types. However, he fears that drug companies will price vaccines out of the reach of developing countries. Researchers at the Krebsforschungszentrum continue to look for cheaper alternatives, especially one that could be delivered through a nasal spray rather than by injection.

The papilloma vaccine is not the first to protect against cancer. "Hepatitis B in my opinion is the first anticancer vaccine, although it was developed to prevent the symptoms of acute Hepatitis B," zur Hausen points out.

Taiwan introduced a Hepatitis B vaccination programme in 1985. A study in 1995 showed a dramatic reduction in Hepatitis B infection amongst vaccinated children, and Taiwan is beginning to see a reduced number of liver cancers in teenage children.

zur Hausen believes that vaccines could substantially reduce the risks of cervical cancer over the next 20 years, and that targeted chemotherapy will be effective for those who already have invasive cancer.

He has spent his working life finding the evidence to show the role of papilloma virus, and although the road has been long and tortuous, he does not regret staying with it.

"Some of my colleagues think I am a bit stupid because I followed one thing for the whole of my career – the infectious agents for carcinogens. Many of those who worked with me in the early days changed to do something else. I believe that these chronic diseases demand a persistent involvement from the scientific side."

zur Hausen is preparing a major text book on infectious causes of cancer. The story he has been telling for 30 years is now broadly accepted as correct, but he believes there is more explaining to do. "I am relatively quiet and not an immediately aggressive person," he says, "but I think I can persuade people to do what is necessary".

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