SEX, CANCER and a VIRUS

EMERGING THREAT

Rates of head and neck cancer (purple) have risen — and they are set to grow further. An increasing proportion of cases is caused by human papillomavirus (HPV, yellow). At the same time, rates of cervical cancer (red; nearly all caused by HPV) have declined, owing to increased screening.

*Estimate based on clinical observations

SOURCE: M. GILLISON/A. K. CHATURVEDI *ET AL. J. CLIN. ONCOL.* **29**, 4294–4301 (2011)

Human papillomavirus is causing a new form of head and neck cancer— leaving researchers scrambling to understand risk factors, tests and treatments.

BY MEGAN SCUDELLARI

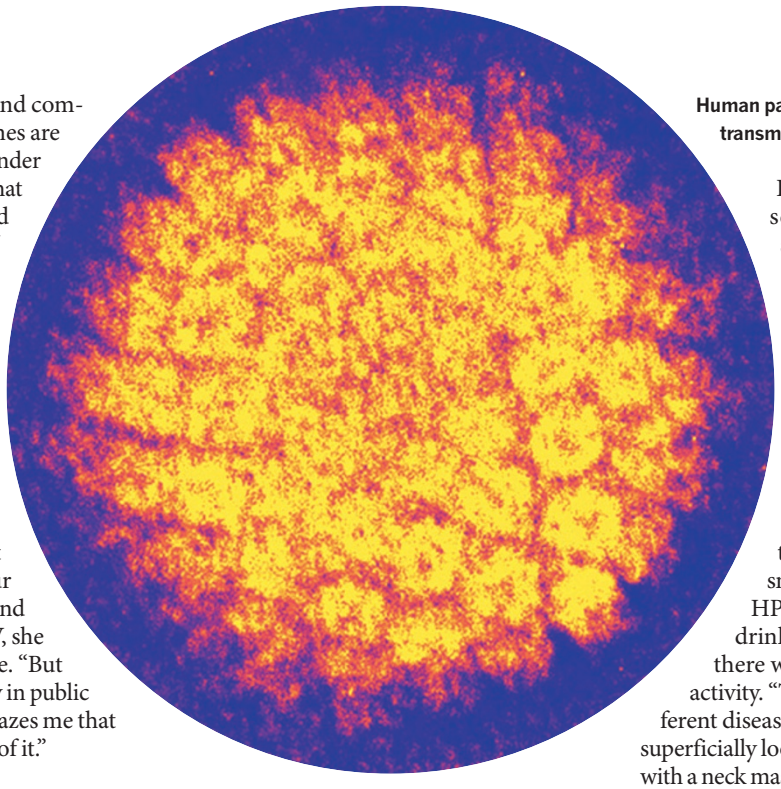
On a sunny day in 1998, Maura Gillison was walking across the campus of Johns Hopkins University in Baltimore, Maryland, thinking about a virus. The young oncologist bumped into the director of the university's cancer centre, who asked politely about her work. Gillison described her discovery of early evidence that human papillomavirus (HPV) — a ubiquitous pathogen that infects nearly every human at some point in their lives — could be causing tens of thousands of cases of throat cancer each year in the United States. The senior doctor stared down at Gillison, not saying a word. "That was the first clue that what I was doing was interesting to others and had potential significance," recalls Gillison.

She knew that such a claim had a high burden of proof. HPV was known to cause cervical cancer and small numbers of genital cancers, but no other forms. So Gillison started a careful population study comparing people with cancer to healthy individuals. Over seven years, she recruited 300 participants, collected tissue samples, and never once looked at the data. "My policy, when doing a study, is that we wait until all the data are in, and do all the analyses at once," says Gillison, who is as careful as she is blunt. "I don't know anything until the data tell me."

Only in 2005 did Gillison finally sit down with a doctoral student to analyse the data. Within an hour, the fruits of those years of labour popped up on the computer screen: people with head and neck cancer were 15 times more likely to be infected with HPV in their mouths or throats than those without[1]. The association backed up some of Gillison's earlier work, which showed[2] how HPV DNA integrates itself into the nuclei of throat cells and produces cancer-causing proteins. Gillison leapt from her chair and began jumping up and down. "The association was so incredibly strong, it made me realize this was absolutely irrefutable evidence," she says.

Since then, she and a network of other researchers have amassed a mountain of evidence that HPV causes a large proportion of head and neck cancers, and that these HPV-positive cancers are on the rise. The finding has been "a paradigm-shifting realization in the field", says Robert Ferris, chief of the division of head and neck surgery at the University of Pittsburgh Cancer Institute in Pennsylvania.

The medical community is struggling to come to grips with the implications. There is currently no good screening method for HPV-caused



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Human papillomavirus, seen in a coloured transmission electron micrograph.

cancer in the head and neck, and commercially available HPV vaccines are still prescribed only to people under the age of 26, despite evidence that they could prevent head and neck cancer in all adults. Plus, if HPV can get into the mucous membranes of the mouth and throat, where does it stop? There are hints that HPV is a risk factor for other, even more common, types of cancer, including lung cancer.

For now, researchers and doctors need to learn more about how HPV causes cancer, and how best to prevent and treat it, says Gillison. "Our clinics are flooded" with head and neck cancers triggered by HPV, she says, vexation clear in her voice. "But though I talk about it constantly in public settings and the lay press, it amazes me that it's often as if no one has heard of it."

NEW THREAT

James Rocco, director of head and neck molecular oncology research at Massachusetts General Hospital in Boston, remembers the first signs that something was changing. Until the late 1990s, most cases of cancer in the back of the throat (the oropharynx) could be blamed on alcohol and tobacco use: the majority of Rocco's patients were men around 50 years old, who had been smoking and drinking for 30 years. But then 40-year-old marathon runners and people in otherwise good health began to trickle — then stream — into his office. And when treated with chemotherapy and radiation, these people seemed to have better survival rates than the other head and neck cancer patients.

There were also irregularities in the laboratory. When biopsied, the site of the cancer was slightly different in this healthier cohort: instead of beginning on the surface of the tonsil as normal, tumours seemed to start deep in tonsil crevices. And more and more of the tumours lacked mutations in a protein called p53 — then considered a hallmark of oropharyngeal cancer. "We kind of knew we were dealing with something different," recalls Rocco.

Gillison started pursuing the issue in 1996, after a passing comment by a colleague. Keerti Shah, a molecular microbiologist at the Johns Hopkins Bloomberg School of Public Health, had mentioned research in Finland that had identified HPV in a cell line developed from an oropharyngeal tumour[3]. As Shah and Gillison walked around campus one day, they talked about the finding. Was it an isolated case? Had HPV contaminated the sample? Or, as Shah suspected, could HPV cause some cases of head and neck cancer?

Gillison went straight to her office to do a literature search. She began analysing tumour samples from the Head and Neck Cancer Center at Hopkins and found HPV in about 25% of them. She used multiple techniques to be sure that positive results were not attributable to laboratory contamination. She looked for the virus in early, middle and late stage tumours. HPV was not just present; she found that its DNA had infiltrated the tumours and was producing two potent oncoproteins, an indication it was the cause of the cancer. Gillison also profiled people with HPV to learn about the cancer's clinical characteristics, and identified molecular biomarkers that were absent in tumours without HPV. She worked on the project for 18 months, without taking a day off.

If HPV can get into the mucous membranes of the mouth and throat, where does it stop?

She, Shah and their colleagues published their results in 2000 (ref. 2), demonstrating that HPV-positive oropharyngeal cancer is a distinct type of cancer that starts deep in the tonsils, has HPV DNA present in the tumour-cell nuclei but not neighbouring cells, has fewer p53 mutations than HPV-negative cancer, has less association with smoking and alcohol consumption and has better survival rates. But many oncologists were sceptical: some suspected that HPV was just a passenger virus, or that its presence was the result of contamination. Others thought that HPV might be just a risk factor, rather than a cause, for head and neck cancer — one of several ingredients, including drinking and smoking, that when combined together congealed into a cancerous stew.

In 2007, Gillison published her seven-year population study showing the link between oral HPV infection and oropharyngeal cancer[1]; the next year, she released a study[4] showing that HPV-positive and HPV-negative oropharyngeal cancers had completely different risk profiles. People with HPV-positive cancer tended to have had many oral-sex partners, but there was no statistical association with tobacco smoking or drinking; those with HPV-negative cancers were heavy drinkers and cigarette smokers but there was no association with sexual activity. "These were two completely different diseases," says Gillison. "They might superficially look similar — a patient comes in with a neck mass and their throat hurts — but I realized what drove the pathogenesis was completely different in the two cases."

By then, all doubts had faded. In 2007, the World Health Organization's International Agency for Research on Cancer in Lyons, France, declared that there was sufficient evidence to conclude that HPV causes a subset of oropharyngeal cancers. Gillison's research has been "definitive", says Jeffrey Myers, director of head and neck surgery research at the University of Texas MD Anderson Cancer Center in Houston.

Community acceptance came not a moment too soon. The number of oropharyngeal cancers has been growing over the past 30 years: there are now 10,000 cases in the United States each year, a number that is likely to climb to 16,000 by 2030 (see 'Emerging threat'). An overwhelming majority are caused by HPV. Worldwide, cancer centres report that the virus is responsible for between 45% and 90% of oropharyngeal cancers . "In Europe, HPV-positive oropharyngeal cancers have almost quadrupled in number over a period of 10 to 15 years," says Hisham Mehanna, director of the Institute of Head and Neck Studies and Education at the University of Birmingham, UK, who has published a meta-analysis[5] of more than 250 papers on prevalence rates. "Our projection suggests that it's going to continue to increase significantly." Why rates are escalating is unknown, although one suggestion points to increasing numbers of sexual partners.

PROBLEM PROTEINS

It turns out that HPV causes throat cancer in much the same ways as it causes cancer in the cervix. The virus's DNA integrates into human DNA in the nuclei of healthy cells, and uses the cells' machinery to produce two harmful proteins, E6 and E7. These bind to, and shut down,

two important tumour-suppressor proteins, p53 and pRb. Active pRb prevents excessive cell growth; without it, cells proliferate unchecked. Active p53 arrests the cell-division cycle when DNA is damaged, and then either activates DNA repair or initiates cell death. Without p53, a cell replicates wildly even if it has DNA damage.

In cancers caused by HPV, the virus silences p53 but leaves the gene that produces it intact; by contrast, in HPV-negative cancers, the gene is mutated, probably through exposure to carcinogens, and produces an ineffective version of the protein. This may explain why people with HPV-positive oropharyngeal cancer respond better to treatment: early evidence suggests[6] that chemotherapy or radiation may somehow reactivate p53 in HPV-positive cancers, turning the powerful protein back on to fight the tumour.

There are other possibilities. It could be that people with HPV-positive cancer are generally healthier than their HPV-negative counterparts: they tend to be younger, generally don't smoke and are more likely to comply with treatment regimes. Another possibility, supported by a study[7] using sequencing data from 74 head and neck cancers, is that HPV-negative tumours are more heterogeneous than HPV-positive tumours. The cells have many more mutations, and a wider range of them. In an HPV-negative tumour, therefore, "there's more likely to be something in there that will resist therapy", says Rocco, a co-author of the study.

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"These diseases might look similar, but what drove the pathogenesis was completely different."

Maura Gillison

TOXIC TREATMENT

The fact that people with HPV-positive cancer have better outcomes has caused many clinicians, including Gillison and Ferris, to wonder whether these patients should get different treatments. The current standard therapy for oropharyngeal cancer is a combination of cisplatin — a toxic, potent chemotherapy drug — and radiation. This has many potential side effects, including damage to the voice box and throat, which can hinder the ability to speak and swallow. With the younger, healthier HPV-positive patients, who are 58% less likely to die within three years of treatment than HPV-negative patients, clinicians worry about the long-term effects of the treatment, and are exploring techniques including less-toxic chemotherapy regimens.

Researchers are also looking at ways to prevent the disease in the first place. More than 90% of HPV-related oropharyngeal cancers are caused by HPV-16, a particularly dangerous strain and the main cause of cervical cancer. The two vaccines approved to prevent cervical cancer, Merck's Gardasil and GlaxoSmithKline's Cervarix, both protect against HPV-16. In theory, therefore, protection against HPV-positive oropharyngeal cancer is already in doctors' cabinets. A clinical trial of 5,840 women, published this year by researchers at the US National Cancer Institute[8], showed that Cervarix is 93% effective at preventing oral HPV infection in both women with pre-existing cervical infections and those without, none of whom had been previously vaccinated.

A major barrier stands in the way of official approval for using the vaccine to protect against oropharyngeal cancer: there is not yet a way to prove that it would work. For cervical cancer, doctors test cells taken from the cervix during routine screening, looking for changes that precede the emergence of cancer. Because HPV-positive oropharyngeal cancer arises deep in the tonsil, checks would have to be much more invasive. "In theory, we could detect it, but we would need to do a tonsillectomy on everyone in the vaccine trial," says Gillison. "That's never going to happen."

There may be another way. Mehanna and his colleagues are in the process of analysing the tonsils of 1,250 people who underwent tonsillectomies for non-cancerous reasons. The researchers have identified what they think are pre-malignant lesions in some HPV-positive samples that may represent the earliest stages of the cancer, and could serve as a biomarker. "We're now testing to make sure this pre-malignancy is driven by HPV and is not just random," says Mehanna.

Other concerns and questions linger. For example, scientists have yet to determine whether oral HPV infection comes only from sexual acts that involve contact between the mouth and genitals, or also from other acts including deep kissing. And most people who develop an HPV infection do not get oropharyngeal cancer: about 90% of those who become infected orally clear the infection within two years. No one is sure why.

Researchers are also investigating whether HPV causes other types of cancer. There have been studies of the relationship between the virus and oesophageal cancer, but findings have been inconclusive. Another area of interest is the lung. There, too, tobacco has been the primary culprit for decades, but some 15–20% of lung-cancer cases in men and 50% in women are in people who have never smoked. Doctors have theorized that a virus lies behind them.

The available data are conflicting. One paper[9] in 2001 identified HPV DNA in 55% of 141 lung tumours, compared with 27% of 60 non-cancer control samples. And in 2009, researchers led by Iver Petersen, director of the Institute for Pathology at Jena University Hospital in Germany, conducted a meta-analysis[10] of 53 publications examining 4,508 cases of lung cancer, and concluded that "HPV is the second most important cause of lung cancer after cigarette smoking". They encouraged more research. But many other studies have refuted those observations, including one from Gillison and her colleagues, in which they used sensitive DNA assays to study the lung cancers of 450 patients, and found no HPV (ref. 11).

With head and neck cancer, however, Gillison is optimistic that new knowledge about HPV as a cause of the disease will help physicians to treat it — and eventually to prevent it with a vaccine. "In terms of cancer," she says, "there aren't many populations where we've identified the necessary cause and have a potential solution on the shelf." ■

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1. D'Souza, G. *et al. New Engl. J. Med.* **356,** 1944–1956 (2007).
2. Gillison, M. L. *et al. J. Natl Cancer Inst.* **92,** 709–720 (2000).
3. Syrjänen, K. J., Pyrhönen, S., Syrjänen, S. M. & Lamberg, M. A. *Br. J. Oral Surg.* **21,** 147–153 (1983).
4. Gillison, M. L. *et al. J. Natl Cancer Inst.* **100,** 407–420 (2008).
5. Mehanna, H. *et al. Head Neck* **35,** 747–755 (2013).
6. Xie, X. *et al. Oncogene* http://dx.doi.org/10.1038/onc.2013.25 (2013).
7. Mroz, E. A. & Rocco, J. W. *Oral Oncol.* **49,** 211–215 (2013).
8. Herrero, R. *et al. PLoS ONE* **8,** e68329 (2013).
9. Cheng, Y. W. *et al. Cancer Res.* **61,** 2799 (2001).
10. Klein, F., Amin Kotb, W. F. M. & Petersen, I. *Lung Cancer* **65,** 13–18 (2009).
11. Koshiol, J. *et al. J. Natl Cancer Inst.* **103,** 501–507 (2011).