

BRAIN CANCER

A Viral Link to Glioblastoma?

Circumstantial evidence hints that cytomegalovirus, a common herpesvirus, may play a role in the aggressive brain cancer, but big questions remain

SAN FRANCISCO, CALIFORNIA—Neurosurgeon Charles Cobbs is trying to remove as much of a brain tumor as possible without damaging tissue important for speech. He needs the patient's cooperation, so the anesthesiologist dials down the propofol anesthetic and places a laptop computer on a stand near the patient's head. The 77-year-old man, who has a glioblastoma near the crease that separates the frontal and temporal lobes, slowly wakes up.

Images of familiar objects flash on the screen, and the patient names them while Cobbs electrically stimulates his exposed cortex with a hand-held probe. "Cow, car, hairbrush," the man says. He sounds groggy, but the words are unslurred. Cobbs turns up the current for stronger stimulation and does another pass as the man counts to 50. Satisfied that the tumor can be safely resected, the surgical team here at the California Pacific Medical Center puts the patient back under, and Cobbs scoops out a bloody glob of tissue, most of which goes into a vial to be sent to the lab.

After the surgery, Cobbs is optimistic. Glioblastoma is the worst grade of malignant glioma, a relatively uncommon but deadly class of brain tumors that kills 97% of those diagnosed within 5 years. But Cobbs says the material he removed had the rubbery feel of dead tissue rather than the "grape jelly" consistency of a growing tumor. That would be good news for the patient, and Cobbs thinks it may also bode well for his own unorthodox hypothesis about malignant glioma.

Cobbs thinks the cancer may be caused, or at least spurred on, by cytomegalovirus (CMV), a common herpesvirus. Several years ago, Cobbs reported that malignant gliomas are rife with CMV, and he says work

by other researchers supports his idea that the virus may be a potent promoter of these cancers. His patient has been taking an antiviral drug called Valcyte, made by Roche Pharmaceuticals, in addition to receiving standard radiation and chemotherapy. Cobbs thinks drugs and other therapies that target CMV may extend the lives of patients who otherwise have few options.

The idea is controversial and unproven. Why would such a common virus cause cancer in only a small subset of those infected? And why have test tube experiments so far failed to show that CMV transforms healthy cells into cancerous cells? These and other nettlesome questions beg for answers before Cobbs's theory can gain widespread acceptance.

Yet several researchers and clinicians who attended a meeting Cobbs organized in Boston last October say there is enough circumstantial evidence in support of the idea to merit serious attention. "It's a promising concept, and it gives us hope of new treatment strategies for this horrible disease," says Cecilia Söderberg-Nauclér of the Karolinska Institute in Stockholm, Sweden. She and others have already begun small clinical trials with drugs and immunotherapy directed at CMV.

Counter to the mainstream

At age 45, Cobbs still has the rangy build of a collegiate rower, which he was at Princeton University. Soft-spoken and bespectacled, he doesn't come across as a rebel, although his thinking about malignant glioma has run

counter to the mainstream. Cobbs did research as a medical resident suggesting that these tumors were "boiling cauldrons of inflammatory molecules," and he wondered whether this inflammation might somehow trigger the cancer or help it grow. It wasn't a fashionable idea at the time, and for a young investigator, it was a risky avenue to pursue.

Reading *Surely You're Joking, Mr. Feynman!*, a memoir written by the iconoclastic physicist Richard Feynman, boosted his confidence. Cobbs says the message he took away from the book is that "your job as a scientist is to think for yourself and question what the herd is thinking." Cobbs had a hunch that CMV might be just the sort of virus to lurk around and cause the kind of chronic inflammation he suspected of contributing to cancer. The virus infects for life,

and mouse studies have found that it infects the neural stem cells thought to give rise to glioblastomas. To investigate, he and colleagues tested 27 malignant glioma samples for CMV proteins and genetic material. All were positive, the researchers reported in *Cancer Research* in 2002. Samples of noncancerous brain tissue tested negative.

Not everyone was convinced by the findings. In 2005, a team of researchers at City of Hope Hospital in Duarte, California, reported in *Modern Pathology* that they'd failed to find CMV in glioma samples. "I was kind of distressed and heartbroken," Cobbs recalls. A team at Duke University Medical Center in Durham, North Carolina, wasn't finding CMV in its samples, either. "We called [Cobbs] and said, 'What's the deal?'" says

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—CHARLES COBBS,
CALIFORNIA PACIFIC
MEDICAL CENTER

Delicate operation. Charles Cobbs removes a brain tumor at California Pacific Medical Center.

Duane Mitchell, a neuro-oncologist at Duke. Cobbs suspected that the other researchers were using less sensitive methods and offered to visit both teams to demonstrate his technique. Only the Duke team took him up on it.

Since then, there have been several confirmations. Last February, Mitchell and colleagues reported finding CMV in more than 90% of glioma samples they examined; a team at M. D. Anderson Cancer Center in Houston, Texas, published similar findings a few months later; and Söderberg-Nauclér and colleagues have a forthcoming paper as well. “Without a question, the virus is there,” says Söderberg-Nauclér. But is it a bystander or an agent provocateur?

A suspicious resume

CMV is one of eight human herpesviruses; it infects at least half of the population in developed countries and nearly everyone in developing countries, where poor sanitation and hygiene encourage its transmission. Although it generally doesn't cause problems in healthy adults, CMV is a common cause of birth defects, and it can cause a host of serious problems in immunocompromised people.

How it might trigger or abet cancer is not known, but there's no shortage of possibilities, says virologist Jay Nelson of Oregon Health and Science University in Portland. One way it could do this “is by preventing the immune system from taking the tumor out,” he posits, because CMV is adept at manipulating signaling molecules on cell surfaces that would otherwise flag infected cells for destruction.

Another possibility is that the virus helps sustain tumors by promoting angiogenesis, the growth of new blood vessels. Nelson's lab reported last year that CMV-infected heart cells churn out a slew of growth factors and other compounds that spur angiogenesis.

The virus also makes proteins that interfere with cellular machinery that normally prevents tumors, says Robert Kalejta, a cancer biologist and virologist at the University of Wisconsin, Madison. In 2003, Kalejta and colleagues reported that a CMV protein called pp71 helps break down retinoblastoma protein (Rb), a potent tumor suppressor. More recently, they identified a CMV enzyme called UL97 that inactivates Rb

(*Science*, 9 May 2008, p. 797). “Clearly, these are two proteins that can do what's required” to transform healthy cells into tumor cells, Kalejta says. “The question is, are they really doing that in these cancers?”

Kalejta says he's intrigued but not yet convinced. He notes that unlike proven cancer-causing viruses such as human papillomavirus and Epstein-Barr virus, CMV has not been shown to transform healthy cells into cancerous cells. The lack of direct evidence that CMV can cause cancer remains an important caveat, Kalejta says.

Liliana Soroceanu, a neuroscientist who collaborates with Cobbs at the California Pacific Medical Center, hopes to gain clues about how CMV might contribute to malignant glioma by culturing cells from tumor tissue Cobbs has removed from patients. She plans to use a CMV gene chip developed by Nelson's team to examine which genes the virus turns on in infected glioma cells—and to see whether turning those genes off, using RNA interference, for example, slows the growth of cultured tumors.



Partners in crime? Cobbs suspects that malignant gliomas like this one may be aided by cytomegalovirus.

Beside stories

Evidence of a link between CMV and glioma has come from the bedside as well as the lab bench. One serendipitous finding comes from a clinical trial with glioblastoma patients run by Robert Prins and colleagues at the University of California, Los Angeles. They have been testing a vaccine intended to train patients' immune systems to attack their tumors. To do this, they grow immune cells from each patient in culture, expose them to proteins from that patient's tumor, and inject them back into the patient.

As it happens, one patient responded to the vaccine by mounting a massive immune response against a CMV protein, the

researchers reported last July in *The New England Journal of Medicine*. That patient has now survived nearly 6 years without a recurrence of the cancer, longer than 99% of patients who receive standard care, Prins says. (All patients in the trial received standard care—surgery, radiation, and chemotherapy—in addition to the vaccine.) Although it's impossible to know if the vaccine is the reason, given the slim odds of surviving that long with glioblastoma, Prins thinks it's highly likely.

At Duke, Mitchell and colleagues are pursuing a similar approach more tightly focused on CMV. They are vaccinating glioblastoma patients with immune cells exposed to CMV proteins. The trial is intended to assess the safety of the treatment, but there are signs that it is having the desired effect. At November's meeting of the Society for Neuro-Oncology, the researchers reported that the vaccine had enhanced immune responses against CMV, and the 21 patients in the trial had a median survival time of more than 20 months. The median survival time so far for such patients is typically less than 15 months, Mitchell says.

At the Karolinska Institute, Söderberg-Nauclér and colleagues have just completed a 2-year trial with Valcyte, the antiviral drug Cobbs's patient received. (Cobbs prescribed the drug off-label, not as part of a clinical trial.) The 42 patients with glioblastoma began taking Valcyte after surgeons had removed their tumors and researchers confirmed that the tumors were infected with CMV. Researchers used magnetic resonance imaging to look for signs that tumors grew back. The study is double-blinded, so the researchers don't yet know which patients received the drug and which received a placebo. Even so, Söderberg-Nauclér is optimistic.

“We have patients that looked extremely bad at the beginning that have survived 2 years,” she says. The trial concluded just before Christmas, and the team should know soon whether those patients received the drug—or just a remarkable stroke of good luck.

Cobbs says he's hopeful that if the Swedish trial goes well, Roche will fund a larger one. He insists he doesn't want to hype the evidence for a link between CMV and glioma but says he's gratified that other researchers are starting to take the idea seriously. “It may be a house of cards, but my gut tells me it might pan out to be something big time.”

—GREG MILLER