CANCER GENETICS

Link to Hereditary Melanoma Brightens Mood for *p16* Gene

Since its discovery a year ago, the gene for the protein known as p16 has given researchers as many mood swings as a teenager in love. Euphoria soared this spring with reports that *p16* might be responsible for some cases of hereditary melanoma, a skin cancer that is very dangerous if not diagnosed early. Not only that, the work also suggested that the gene might be an important tumor suppressor, implicated in many cancers besides melanoma.

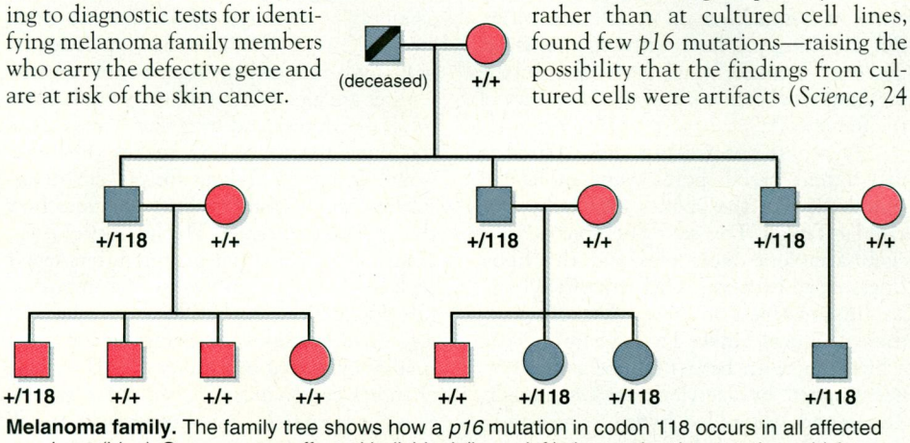
Within weeks, though, the mood turned into something resembling the aftermath of a bad first date. The early results came mainly from tumor cells in lab culture, and new findings from primary tumor samples suggested that *p16* (now also known more formally as the *CDKN2* gene) is not a general tumor suppressor. And even *p16*'s status as an inherited melanoma susceptibility gene remained murky, as the family studies needed to prove that case had not been completed.

Now, the mood surrounding *p16* is brightening again. Two groups report in the September issue of *Nature Genetics* that they have identified mutations in the *p16* gene that are associated with melanoma in some—although not all—families. These results suggest *p16* is indeed contributing to development of hereditary skin cancers. "It's going to be an important gene for cancer susceptibility in those families," says cancer gene expert Stephen Friend of Massachusetts General Hospital in Boston. The question of whether *p16* is also a more general tumor suppressor remains open—although new evidence links it to pancreatic and esophageal cancers. But even if it's only a familial melanoma gene, knowledge of *p16* mutations would still be useful, leading to diagnostic tests for identifying melanoma family members who carry the defective gene and are at risk of the skin cancer.

The trail of evidence that linked *p16* to hereditary melanoma was first picked up about 2 years ago when genetic linkage studies from a team led by Lisa Cannon-Albright of the University of Utah School of Medicine in Salt Lake City and Mark Skolnick, who holds a joint appointment at the university and at Myriad Genetics Inc. in Salt Lake City, indicated that segment p21 of chromosome 9 contains a melanoma susceptibility gene.

Following up on that observation, Skolnick, Alexander Kamb of Myriad, and their colleagues began scouring 9p21 in cultured melanoma cell lines, looking for a region that was consistently deleted, which would suggest that it contains a gene that normally suppresses tumor formation. The Utah team found such a region, and when they cloned and sequenced it, out popped the *p16* gene, which had been cloned only 6 months before by David Beach's group at Cold Spring Harbor Laboratory on Long Island (*Science*, 15 April, pp. 344 and 436). The new find was a particularly interesting suppressor candidate, because Beach and his colleagues had shown that its protein product inhibits a key regulatory enzyme in the cell cycle, the complex machinery that tells cells to divide. Its loss might therefore push cells into the uncontrolled division of cancer.

Studies on cultured cells supported the idea that *p16* was an important tumor suppressor, because the Kamb group found that the gene is deleted or mutated, not just in melanoma cells, but in a wide variety of other tumors as well, including breast, lung, and bladder cancer. But that finding was quickly called into question when other researchers, looking at primary tumors rather than at cultured cell lines, found few *p16* mutations—raising the possibility that the findings from cultured cells were artifacts (*Science*, 24 June, p. 1846 and 15 July, p. 415).



SOURCE: NICHOLAS DRACOPOLI/NIH

Melanoma family. The family tree shows how a *p16* mutation in codon 118 occurs in all affected members *(blue)*. One as-yet-unaffected individual *(lower left)* also carries the mutation, which presumably came from the deceased first-generation male (+ indicates normal gene).

Even if they were genuine, proving that *p16* is the melanoma susceptibility gene requires more than cell culture studies: Researchers also need to show that mutations in the gene are consistently found in melanoma patients and not in unaffected members of families with hereditary melanoma.

That's where the new work comes in. One group, led by Nicholas Dracopoli of the National Center for Human Genome Research at the National Institutes of Health (NIH), has identified six *p16* mutations that track with melanoma in nine of the 15 families in their study with possible linkage to 9p21. In those families, 33 of the 36 members who have melanoma carry a *p16* mutation, says Dracopoli. While the NIH group also found mutations in 15 unaffected members, about half of those people may be too young to have developed melanoma. In addition, previous experience has shown that not all carriers of the melanoma susceptibility gene go on to get the cancer. Summing up, Dracopoli says: "The association is really very clear. I think [*p16*] clearly is the melanoma gene."

The results from the other group, which includes Kamb, Skolnick, and Cannon-Albright, are less clear-cut. These researchers found melanoma-associated *p16* mutations in only two of 11 families. Still, the Utah workers are encouraged by the current results, as the results of the two groups are in general agreement; the two mutations the Utah workers spotted, for example, are among those identified by the Dracopoli group.

Taken together, the two groups' work shows that "*p16* is *a* melanoma susceptibility gene," Skolnick says. Nevertheless, he acknowledges, "it's also clear that a number of families linked to the [9p21] region haven't been explained." One possible reason, he says, is that both groups have looked only at the protein-coding regions of the *p16* gene. In the families whose tumors haven't yet been explained, the mutations may lie outside the protein-coding area in the regions that regulate the gene's activity.

Still, there is another possibility: that there is another, unidentified, tumor suppressor gene on 9p21 that may be involved in the hereditary melanomas not caused by *p16* mutations. Indeed, some researchers think that such a gene exists and that it is a more general contributor to cancer development than *p16*. Their reasoning is based partly on the fact that deletions suggestive of a tumor suppressor have been found at 9p21 in several types of cancers in addition to melanoma. David Sidransky of Johns Hopkins University School of Medicine in Baltimore, whose group was among those that didn't find *p16* mutations in most nonmelanoma primary tumors, favors this idea. "I think that the [*p16*] gene is a tumor suppressor and it's

involved in some cancers, such as melanoma," he says, "but it's not 'the biggie'" everyone is looking for on 9p21. Kamb, meanwhile, is sticking to his guns about *p16*. "I've never doubted that this gene was a major player in cancers of many kinds," he says.

Kamb has other recent evidence in his favor: studies suggesting that loss or inactivation of *p16* contributes to the development of at least two additional cancers. Takahiro Mori, Yusuke Nakamura, and their colleagues at the Tokyo Cancer Institute and Tohoku University School of Medicine in Sendai report in the 1 July issue of *Cancer Research* that the gene is mutated in 14 of 27 esophageal cancers, but not in the surrounding normal tissue. And in the September *Nature Genetics*, a team led by Scott Kern of Johns Hopkins University School of Medicine reports finding mutations or deletions of the gene in about 75% of the human pancreatic cancers they've examined. "It's a tumor suppressor, and it's important," Kern concludes. If results like those continue to flow in, the *p16* mood may yet swing back to euphoria.

–Jean Marx

NEUROSCIENCE

Alzheimer's: Could There Be a Zinc Link?

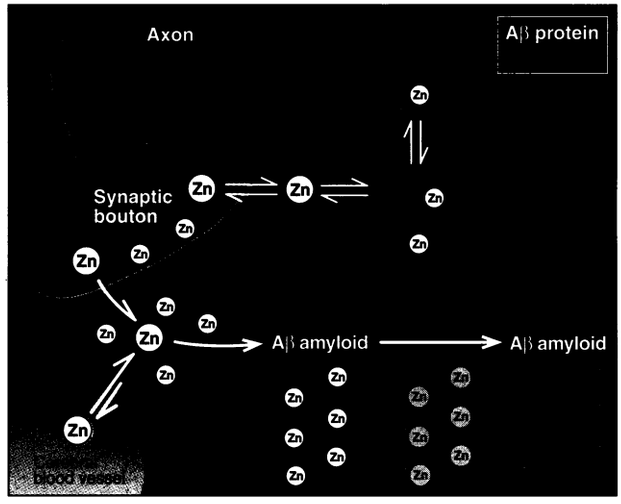
The effort to understand the causes of Alzheimer's disease has been so long and so frustrating that researchers have resorted to some unusual lines of inquiry. In 1991, for example, motivated by reports that zinc improves mental alertness in the elderly, a team in neuroscientist Colin Masters' lab at the University of Melbourne gave zinc supplements to a handful of Alzheimer's patients and age-matched controls. The results, however, were disastrous: Within 2 days, the cognition of the Alzheimer's patients deteriorated markedly.

To avoid further harm, Masters' team immediately halted the study, without having a chance to learn much about the biochemistry behind the disturbing results. Now, however, some researchers have taken a different approach—using a test tube—and have offered direct biochemical data hinting at a zinc-Alzheimer's connection. On page 1464 of this issue, Ashley Bush, a postdoc working with Rudolph Tanzi of Harvard University's Massachusetts General Hospital, and his co-workers report that zinc ions can cause one form of the Aβ protein (Aβ) to form clumps resembling the amyloid plaques found in the brains of Alzheimer's patients. Bush, a former graduate student in Masters' group, says, "We believe this information is going to be very important to people who are trying to develop strategies either to prevent or reverse amyloid formation."

Other researchers, though they hail the intriguing nature of this work, stress that it's very preliminary—and only from the test tube, which may not reflect real-life conditions. "It's a very interesting finding," says Alzheimer's researcher Steven Younkin of Case Western Reserve University in Cleveland, "but it's clear that it in no way proves zinc is the cornerstone of Alzheimer's. It's more in the realm of raising a very intriguing possibility." Younkin and others also cautioned that it's far too early for anyone to think about removing zinc from their diet.

Tanzi's group began the current work in 1992 after Bush joined the lab. Scientists had known Aβ was the main constituent of Alzheimer's plaques, and had recently shown that Aβ is present in a soluble form in cerebrospinal fluid. What, then, made the protein clump together in plaques only in the brain? Tanzi's team, mindful that zinc is important in the brain, decided to see how readily zinc and other transition metals could bind to Aβ and prompt formation of amyloid clumps. Zinc, they found, had by far the most significant effect.

At low concentrations such as those found in cerebrospinal fluid, zinc bound to Aβ without causing it to clump and precipitate out of solution. But when zinc was present at a concentration just above that, the peptide suddenly clumped. The clumps were similar in size to naturally occurring amyloid plaques, and they looked the same when stained and viewed with polarized light. Zinc did not have this effect on Aβ produced by rats, which do not develop amyloid plaques as they age—and whose Aβ differs from the human version by three amino acids. In the brain, Tanzi says, it's quite plausible that if the strict regulation of zinc broke down, sufficient concentrations of zinc could contact Aβ and form it into clumps.



Metal in the mind. If zinc is involved in the formation of amyloid plaques, high concentrations of the metal at the end of the axon—the synaptic bouton—could be the trigger, as shown in the pathway at left.

Critics, however, have a number of questions about the paper's conclusions and about the zinc-amyloid hypothesis in general. Carl Cotman of the University of California at Irvine notes that while the Tanzi study used free zinc ions in solution, in the brain zinc is bound to other proteins and may not be available to react with Aβ. And zinc may not be needed at all: At sufficiently high concentrations, Aβ can aggregate spontaneously, and Younkin's group recently reported that some Alzheimer's patients have elevated levels of a longer form of the protein that self-aggregates at a high rate in vitro (*Science*, 27 May, p. 1336).

In addition, Zaven Khachaturian, who heads the National Institute on Aging's Alzheimer's program, cautions that the long-debated question of whether Aβ amyloid is really a key element in causing Alzheimer's or whether the plaques are a secondary consequence of other disease processes is still very much up in the air (*Science*, 4 September 1992, p. 1336). "There are many major, gaping holes in the amyloid hypothesis," he warns.

Still, the new zinc data are important, says Alzheimer's researcher Dennis Selkoe of Harvard University. He and others would now like to see similar experiments done with solutions that more closely mimic the environment of the brain—tests the Tanzi group is already pursuing.

But while tests continue, researchers—including Tanzi—worry about the public's reaction if the results are overplayed in the media. A decade ago, they recall, research proposing a link between Alzheimer's and aluminum—a link now regarded as highly questionable—caused many people to throw aluminum and Silverstone-coated pans in the garbage. Tossing out cookware might be expensive, but it's not dangerous. But removing zinc, a metal that is important to health, from one's diet could have serious repercussions.

–Jocelyn Kaiser