NEWS

TSC genes required for axon formation, study says

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Mutations in the two genes that cause the disease tuberous sclerosis complex, or TSC, interfere with the normal formation of axons, the long and thin strands that conduct electrical signals between brain cells, researchers contend in a report in *Genes and Development* [1].

The paper also shows that in mouse models of TSC, the cancer drug rapamycin can normalize axon formation, providing further evidence for its potential as a treatment for TSC and autism.

TSC is a rare disorder that is characterized by the presence of benign tumors all over the body; about half of all individuals with TSC are on the autism spectrum.

Mutations in the TSC1 or TSC2 genes have been known to cause the disorder for over a decade, but not much is known about the biological mechanism that causes these mutations to wreak havoc in the brain.

In the study, researchers used genetic engineering techniques to both suppress and over-express TSC1 and TSC2 in embryonic mouse brain cells.

"TSC 1 and TSC 2 are crucial," says **Mustafa Sahin**, a neurobiologist at Children?s Hospital Boston and a member of the research team. "If you don't have TSC1 or TSC2, you get multiple axons, not one axon. And if you over-express TSC1 or TSC2, you don't get an axon at all."

These multiple axons may be what's causing the characteristic benign tumors ? called tubers ? and the abnormally shaped ‘giant cells? in the brains of people with the disease, Sahin says.

Previous research on the role of TSC genes had focused on later stages of brain development, when synapses form.

"What's exciting here is that these researchers looked earlier in development, to see what function

TSC1 and TSC2 had just after the neurons were born," says neuroscientist **Jill Wildonger** of the University of California, San Francisco.

That's important for individuals with the disease, she says, because their tubers probably form during these earlier stages.

"We're now better understanding the function of TSC1 and TSC2 at multiple stages in neural development," Wildonger says. "All of these factors along the way affect how neurons communicate with each other ? or miscommunicate."

Big leap:

Others are more cautious about the results, noting that mouse models of the disease are a poor substitute for human studies.

Individuals with the disease have one mutated copy of the TSC1 or TSC2 gene in every cell, but not all of these cells go on to form tubers.

“You can have areas in the brain that look relatively normal, and others relatively abnormal,” explains **Peter Crino**, director of the Tuberous Sclerosis Clinic at the University of Pennsylvania Medical Center. Crino and others say the cells that become tubers are those in which the other copy of the TSC gene also develops a spontaneous mutation.

In contrast, mice that lack the TSC genes don?t produce any tubers; the genetic mutations affect their entire brain.

One clue that human tuber cells have multiple axons lies in a protein the tubers produce called SAD kinase. Sahin?s team found SAD kinase in the mouse TSC knockout cells that produce multiple axons. "So we think it's one of the properties of the tubers, that they have multiple axons and abnormal connectivity," Sahin says.

Because tissue samples from people with TSC are scarce, however, nobody has proven that giant cells or tuber cells have multiple axons. Even in the available samples, it's difficult to determine which projections in the tuber cells are axons and which are dendrites, the fibers that receive nerve signals. “It's a very difficult experiment to do, technically," Sahin notes.

Multiple axons could be one explanation for the abnormally shaped tuber cells, but so could abnormal migration of cells during the embryonic development of the cortex, or a defect in the production of trophic factors ? proteins that help a neuron form connections with its neighbors ? notes Crino.

“If you look in tubers of patients, there?s a markedly disorganized cortical architecture,” says

Crino. “Whether this is due to a disruption in axonogenesis remains to be seen.”

Rapamycin results:

The new work bolsters the idea that TSC could be treated with rapamycin, a drug approved by the US Food and Drug Administration a decade ago to treat kidney transplant rejection.

TSC1 and TSC2 are dynamic parts of a biochemical pathway ? called phosphatidylinositol-3-kinase (PI3-kinase) ? that most cell types rely on for cell growth, survival and programmed cell death.

Rapamycin blocks the PI3-kinase pathway downstream of TSC1 and TSC2, so researchers guessed that giving rapamycin to TSC patients would cancel out the effects of TSC1 or TSC2 malfunction.

They were right. In January, researchers from the Cincinnati Children's Hospital **reported** that when TSC patients were given rapamycin over one year, their tumors shrunk. However, their tumors returned a year after the therapy was stopped[2].

In the following few months, rapamycin?s effects were demonstrated in mice, too. In April, Michael Wong and colleagues from Washington University found that rapamycin, when given prenatally to TSC-knockout mice, prevents epilepsy[3].

In May, Sahin and colleagues found that treating these mice with rapamycin reduces many of their neuronal abnormalities[4]. And in August, some researchers from that group found that rapamycin also reverses learning deficits in these TSC knockouts[5].

In the latest study, the researchers reported that rapamycin prevents neurons that lack TSC genes from developing multiple axons.

Sahin is setting up a multi-center clinical trial to test whether rapamycin affects the intelligence quotient, spatial memory or language development of individuals with the disease. He hopes to start the trial within the next year. The trial will also look at whether rapamycin treatment affects the patients' autism diagnoses.

Rapamycin offers several advantages over the standard invasive treatments for TSC. The drug specifically targets the PI3-kinase pathway, and is already FDA-approved and safe.

Still, some TSC experts caution against making too much of the implications for humans of these results before the clinical trials have been completed.

“Right now, you certainly could not say that we could extrapolate from this data to anything at the bedside,” says Crino. Even if rapamycin is shown to be beneficial in some humans with TSC, he

adds, clinicians should be wary of its primary side effect: it suppresses the immune system.

“The key issue for rapamycin is stratification of appropriate patients to be treated,” Crino says. “Should we give the medication to anyone with TSC? To individuals with just kidney disease or just brain disease? Should we only target infants who are early on in the course? We don?t know the answer to any of those questions yet.”

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