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Researchers Closer to Understanding Tuberous Sclerosis

Researchers have new evidence that may help explain how two genes that are mutated in patients with tuberous sclerosis complex, a genetic disorder that produces widespread benign tumors in the brain, skin, lungs and kidneys, contribute to regulating cell growth and organ size.

In an article published in the May 4, 2001, issue of the journal *Cell*, Howard Hughes Medical Institute investigator Tian Xu and colleagues at Yale University School of Medicine report that the fruit fly protein Tsc1 is responsible for maintaining normal cell size. The fly protein is homologous to TSC1, which is one of two human proteins that malfunctions in patients who have tuberous sclerosis complex.

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— Tian Xu

Xu and colleagues Christopher J. Potter and He Huang also showed that Tsc1 and Tsc2 interact with each other and are likely to be part of the insulin-signaling pathway. The discoveries suggest new molecular targets for therapies designed to treat tuberous sclerosis complex. Furthermore, since the proteins are also part of the insulin-signaling pathway, they may be attractive targets for novel therapies for type 2 diabetes, said Xu.

"Researchers have long known that in humans, mutations in the genes for either TSC1 or TSC2 cause this dominant inherited disorder," said Xu. "And, they have known that the two proteins normally bind to one another in the regulatory process. While it was known that the various mutant proteins were all truncated and didn't bind to each other, there was no clue how that lack of binding caused tuberous sclerosis and how the disorder could be treated."

Xu and his colleagues began their studies by creating genetic mosaic flies. The genetic mosaic flies were originally developed as a way to screen for gene mutations that affect growth. Creating mosaic flies allows researchers to identify gene mutations that might be missed if mutations result in early lethality. In generating mosaic flies, the researchers first use chemicals to induce genetic mutations in the flies. They next examine the normally well-ordered pattern of cells in the flies' eyes for the telltale signs of overgrowth that may characterize mutations in genes that control cell growth.

"This technique mimics the tumorigenic process in humans, because like humans, these flies are chimeric—they have only a few somatic cells that have mutated tumor suppressor genes or oncogenes," said Xu.

In screening mosaic flies for mutations, Xu and his colleagues discovered one mutant that exhibited an abnormal growth of eye cells that resembled the increase in cell size seen in patients with tuberous sclerosis complex. In humans and flies, these cells grow to roughly three times their normal size, which causes an increase in organ size and tumor development.

In these overgrown eye cells, the scientists found the same type of mutation in *Tsc1* that is found in patients who have tuberous sclerosis complex. As in humans, the mutation in the fly *Tsc1* gene caused larger cell growth, cell proliferation and increased organ size.

In additional experiments, the researchers also showed that the fly proteins Tsc1 and Tsc2 bound to each other and worked in concert just as they do in humans. "While it was important to show that the fly system was like that of humans, the most critical contribution of this study was in identifying the metabolic pathway that these proteins affected," said Xu. The scientists' previous studies had shown that mutations in the molecule dPTEN negatively regulates the insulin signaling pathway – which other researchers had shown to be critical for regulating cell size, cell number and organ size in mammals as well.

However, Xu and his colleagues also found that the mutant Tsc1 produced the same overactivation of the insulin pathway, which resulted in enlarged cells like those seen in the *dPTEN* mutants.

A number of detailed experimental manipulations of regulatory proteins in the insulin pathway revealed that the Tsc1 and Tsc2 proteins fit into that pathway—a finding which could have implications for therapy. In particular, said Xu, their experiments showed that an enzyme called S6 kinase that is part of the insulin signaling pathway could be a highly promising drug target for treating tuberous sclerosis.

"Delineating this pathway has suggested that targeting the S6 kinase, which functions downstream of Tsc1 and Tsc2, may be a potential novel therapy for tuberous sclerosis," explained Xu. "We previously had no clue about the affected pathway, and now suddenly we know where to target drugs for this disorder."

Another byproduct of the work, said Xu, is that it may help patients with type 2 diabetes. People who have type 2 diabetes usually don't make enough insulin to metabolize glucose properly. In humans, TSC1 and TSC2 both negatively regulate insulin signaling, so disrupting their function may prove to be a useful treatment for type 2 diabetes, said Xu. "The insulin-signaling pathway is conserved from flies to humans, so these studies suggest that in patients with type 2 diabetes, the TSC1 and TSC2 complex might be a target for therapeutic drugs," said Xu. By using drugs to thwart the binding of the two proteins selectively, explained Xu, one might be able to activate the insulin signaling pathway without producing the symptoms of tuberous sclerosis as a side effect.

Finally, said Xu, finding that mutations in Tsc1 affect organ growth might have implications for understanding fundamental aspects of tumor growth. "In trying to understand tumorigenesis, a great deal of attention has been paid to the proliferation of cells as an underlying cause," said Xu. "But our studies of these regulatory proteins suggest that deregulation of organ size may be another important step in tumorigenesis."