Antioxidant controls spinal cord development

September 18 2009

Researchers at the Johns Hopkins School of Medicine have discovered how one antioxidant protein controls the activity of another protein, critical for the development of spinal cord neurons. The research, publishing this week in *Cell*, describes a never-before known mechanism of protein control.

"This is the first time we've seen this type of chemical reaction control neuronal differentiation," says Shanthini Sockanathan, Ph.D., an associate professor at the Johns Hopkins Solomon H. Snyder Department of Neuroscience. "And it's probably not specific for motor neurons that we study, but also for development of a wide variety of neurons."

Previous research had shown that the GDE2 protein can cause immature cells in the spinal cord to differentiate into motor neurons, the nerve cells that connect to and control muscle contraction. Too little GDE2 causes motor neurons to not develop, while too much GDE2 causes them to develop too quickly, depleting progenitor pools.

"We reasoned that there must be tight control of GDE2 so we set out to look for the regulator by looking for other proteins that can bind to GDE2," says Sockanathan.

Using biochemical approaches to isolate all proteins that normally bind to GDE2 in the developing spinal cord, followed by proteomic analysis to identify all binding proteins, the research team found a few hundred proteins. One, Prdx1, had been reported by others to have tumor-suppressing abilities, which caught Sockanathan's eye for further

investigation.

The team first asked if the Prdx1 protein can affect motor neuron development by removing it from developing spinal cords of chick embryos. Embryos lacking Prdx1 showed loss of motor neurons similar to that seen in embryos lacking GDE2, suggesting that indeed Prdx1 is somehow involved in motor neuron development.

To figure out how Prdx1 and GDE2 interact to cause immature cells to develop into motor neurons, the team mutated the proteins and examined how the mutations affect the cells. Mutations that prevent the two proteins from binding resulted in no motor neurons. Similarly, mutations that disrupt the enzyme abilities of GDE2 and Prdx1 also resulted in no motor neurons. In fact, only when GDE2 and Prdx1 can bind each other and work as enzymes do motor neurons develop.

"So we thought maybe the antioxidant enzyme activity of Prdx1 is doing something to regulate GDE2 function," says Sockanathan. Her team then looked into what already was known about Prdx1's enzyme activity. They found that bacteria and yeast versions of Prdx1 are able to help alter certain chemical bonds in proteins that form between specific amino acids that contain so-called sulfhydryl or "-SH" groups.

That led them to reexamine the GDE2 protein for sulfhydryl groups. As it turns out, they found 4 in GDE2: Three are close together and one is clear on the other end of the protein. They first performed some biochemistry experiments to determine whether these sulfhydryl groups can form disulfide bonds—they can. Then, two at a time, the researchers engineered mutations to replace each -SH-containing amino acid in GDE2 and asked if the mutated protein could still bind to Prx1. They found one combination of mutations that did not behave the same as the unmutated control, leading them to conclude that Prx1 must break the chemical bond between those two specific amino acids.

"We think that Prx1 breaks this bond in GDE2, activating it to promote motor neuron differentiation," says Sockanathan. "This suggests a new general control mechanism that regulates when cells divide and when they differentiate. We're excited to see how widespread it might be."

Source: Johns Hopkins Medical Institutions