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Molecule tells key brain cells to grow up, get to work: study

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About four out of every 10 cells in the brain are so-called oligodendrocytes. These cells produce the all-important myelin that coats nerve tracts, ensuring fast, energy-efficient transmission of nerve impulses. Mixed among them are proliferating but not particularly proficient precursor cells that are destined to become oligodendrocytes when needed but, for now, remain suspended in an immature, relatively undifferentiated state somewhere between stem cell and adult oligodendrocyte.

Stanford University School of Medicine scientists have now identified a molecular master switch that catalyzes these cells' transition to mature, myelin-making mavens. The results may have implications for medical treatment, as defects in this maturation process have been observed in both multiple sclerosis and the most common kind of brain cancers in adults, known as gliomas.

In a study to be published March 10 in *Neuron*, the investigators found that a molecule known as miR-219 is found at high levels only in oligodendrocytes, and that it is both necessary and sufficient to induce their relatively undifferentiated precursors to become functioning adult cells.

"The mechanism responsible for this shifting of anatomical and behavioral gears from precursor to fully functioning oligodendrocyte was a mystery," said Ben Barres, MD, PhD, professor and chair of neurobiology and the study's senior author. "Finding this switch has

allowed us to ferret out several of the molecules it acts on inside cells. And that in turn could open the door to new approaches to treating diseases where oligodendrocyte precursors' failure to mature appropriately plays a role."

A more general question that has puzzled biologists is how cells in one state switch seamlessly to another state. Such a shift implies switching on and off entire banks of genes whose protein products determine a cell's shape, activity and contribution — beneficial or otherwise — to our overall health. The study's results may help to piece that puzzle together.

The specific molecule, miR-219, shown to play a key role in initiating oligodendrocytes' precursor-to-adult transition belongs to a class of molecules known as microRNAs.

RNA molecules are normally thought of as messengers that convey instructions from DNA in the nucleus of animal and plant cells to the surrounding watery zone inside the cell. There, molecular machines that can "read" the messenger RNA's nucleic-acid sequences assemble proteins according to its dictates.

Unlike messenger RNA, microRNA molecules are very short strings of RNA that don't contain instructions for making proteins. While a messenger RNA molecule has to be fairly lengthy to hold all the information necessary for generating a complete protein, a microRNA molecule plays a different role entirely, as Andrew Fire, PhD, Stanford professor of pathology and of genetics and winner of the 2006 Nobel Prize in medicine, discovered.

In the same way that two DNA strands are famously able to form a coordinated double strand when the shapes of the two strands' constituent nucleic-acid sequences are complementary, a microRNA molecule can bind to messenger RNAs when those messenger RNAs'

sequences complement its own. The result is that the messenger RNA's sequence can no longer be read by the cell's protein-manufacturing apparatus, gumming up assembly of the protein it encodes. The binding of microRNA to messenger RNA can even trigger the destruction of the bound complex, setting back the protein-production schedule all the more.

Interestingly, not every bit of a microRNA molecule's sequence has to match its opposite number on a messenger RNA molecule in order for the two to bind. Instead, pairing relies on the recognition of an ultra-short "seed" sequence within the microRNA molecule. "So a single microRNA can affect the activity levels of hundreds of different messenger RNAs," said the study' first author, Jason Dugas, PhD, a research associate in Barres' laboratory.

The study included a collaboration with the lab of Michael McManus, PhD, at the Diabetes Center of the University of California-San Francisco. McManus had generated a customized technique that could measure the relative abundance of different microRNA species in a cell extract.

Meanwhile, the Barres lab is acknowledged worldwide for its prowess in the study of certain types of brain cells. It was Barres who in the 1990s — as a postdoctoral researcher in the Harvard lab of Martin Raff, MDCM, who discovered oligodendrocyte precursor cells — figured out how to refine these precursors to complete purity, keep them alive in a defined culture medium and get them to differentiate into mature oligodendrocytes.

In this latest series of experiments, Dugas, Barres and their colleagues showed that impairing all microRNA production in cells fated to become oligodendrocytes produced both behavioral defects in live laboratory mice and clear anatomical defects (lack of proper myelination) in brain

slices from these mice. Precursor cells cultured in a dish failed to undergo the conversion to adulthood normally triggered by withdrawal of a growth factor, which stimulates precursor cells' proliferation, from the culture medium.

Then, the scientists induced the oligodendrocyte precursor-to-adult transition in normal cells and, using the McManus lab's technology, checked for microRNAs whose levels changed greatly. The amount of one particular microRNA, miR-219, increased by 100-fold at this juncture, Dugas said. That finding has been confirmed in another laboratory that will also publish soon on this subject, Dugas and Barres said.

Staining of brain sections revealed that miR-219 is largely restricted to the brain's white matter — that is, its myelinated regions — making it an excellent biomarker for oligodendrocytes. The researchers also found that delivering a synthetic analog of miR-219 to oligodendrocyte precursor cells deficient in all microRNA generation — and therefore incapable of maturing to myelin-producing oligodendrocytes — partially rescued those cells' ability to mature. Moreover, knocking out only miR-219 function in the oligodendrocyte-fated precursor cells once again prevented them from maturing normally. Adding miR-219 to normal oligodendrocyte precursors in culture, without inducing their differentiation by standard growth-factor withdrawal, increased up to fourfold their likelihood of converting to adulthood.

Finally, the investigators were able to identify several distinct messenger RNAs that were inhibited by miR-219. These messenger RNAs encode proteins that both maintain precursors' proliferative potential and prevent them from becoming full-fledged oligodendrocytes.

"In addition to potential importance for stimulating remyelination in multiple sclerosis, we're especially excited about our findings' potential

significance for glioma, the most common adult brain tumor," Barres said. "There hasn't been any really good treatment for these tumors, in which precursor cells start dividing and dividing and don't differentiate. Why are these cells behaving so abnormally? Maybe this microRNA switch has been downregulated or shut down entirely. Perhaps by introducing miR-219 back into glioma cells, we may actually be able to stop them from behaving like tumor cells." Barres said his lab has entered into a collaboration with another group to test this idea.

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