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Neuroscientists restore cell-to-cell signaling and sociability in autism models

Team uncovers a potential therapeutic approach for patients deficient in a protein called Shank3.

By Alyssa Kneller | Feb 04, 2016

Neuroscientist Ivan Galimberti met a boy with Phelan-McDermid syndrome (PMDS) at a meeting on the rare disease in 2014. The 7-year-old's movements were uncoordinated, he could barely speak, and he wouldn't make eye contact. Like many patients with PMDS, the boy had received a secondary diagnosis of autism spectrum disorder. In this case, doctors knew the cause: a missing copy of a gene called SHANK3. Genes typically come in pairs, but one of the boy's SHANK3 genes had disappeared. As a result, he wasn't producing enough of a structural protein required for stable synapses and optimal communication between neurons.

Galimberti and his colleagues at the Novartis Institutes for BioMedical Research (NIBR) and the Friedrich Miescher Institute for Biomedical Research (FMI) have now uncovered a potential approach for treating such patients. By inhibiting a second protein called CLK2, the researchers were able to restore cell-to-cell signaling between neurons deficient in Shank3. They were also able to restore the sociability of mice deficient in Shank3. These findings appeared online in *Science* on Feb. 4.

"It was incredibly motivating to meet a patient with the exact genetic defect that we study," says Galimberti, who is the senior author and an investigator in Developmental & Molecular Pathways at NIBR. "We worked as quickly as possible to identify and validate new drug targets with him in mind, collaborating with labs at the FMI to accelerate the research."

The discovery might also be relevant to some patients with idiopathic autism, where autism is the primary diagnosis. A 2014 analysis revealed that SHANK3 mutations are present in 0.69% of patients with autism spectrum disorders and up to 2.12% of the cases with moderate to profound intellectual disability. Perhaps these individuals would benefit from CLK2 inhibition.

"From a drug discovery perspective, CLK2 is a tractable target," adds first author Michael Bidinosti, who's a postdoctoral researcher in Galimberti's lab. "It's a protein kinase, and many protein kinases have been drugged successfully in the past."

Protein dragnet

The team came across the target during an experiment that involved proteomics, the large-scale study of proteins. In collaboration with mass spectrometry specialists at NIBR, the researchers analyzed the proteins in rat neurons deficient in Shank3 and compared them with proteins in healthy controls to get a better understanding of what goes awry in Phelan-McDermid syndrome. Specifically, they looked at the amount of phosphorylation of the proteins to gauge their activity. Several proteins in a molecular signaling pathway called Akt/mTORC1 popped out.

In neurons deficient in Shank3, these proteins showed scant phosphorylation, suggesting that Akt/mTORC1 signaling had dropped. The Akt/mTORC1 signaling pathway has previously been linked to autism, so the team was encouraged by the finding.

The proteomics experiment also revealed that the level of the protein kinase CLK2 was twice as high as normal. The researchers conducted additional experiments and determined that this increase was responsible for the drop in Akt/mTORC1 signaling. The connection between CLK2 and the prominent molecular signaling pathway was unexpected, and provides new avenues of investigation in the treatment of Shankopathies.

To the rescue

After identifying CLK2 as a potential target, the researchers used cell and animal models to validate it, work that required deep knowledge of electrophysiology and behavioral assays. Luckily, the NIBR researchers were already meeting regularly with members of the FMI, a research institute affiliated with Novartis and the University of Basel, so they were acquainted with Andreas Lüthi and Pico Caroni, FMI group leaders with the required expertise. Lüthi works on anxiety and has extensive experience with electrophysiology, and Caroni is interested in learning and memory processes with a focus on mouse genetics and behavior.

“Intellectually, our contribution was relatively modest,” says Caroni. “But in order to do these experiments properly, you need the expertise there. You need to know the pitfalls and the tricks. In this case, it was like we were an extension of the NIBR pathways group, working with the team to show the relevance of the initial discovery.”

The team employed several models of Phelan-McDermid syndrome for this phase of the project. First, they knocked down Shank3 in rodent neurons from slices of brain tissue to observe their function in context. The Shank3-deficient neurons produced fewer dendritic spines, projections that are essential for cell-to-cell connectivity. When the team used tool compounds (molecules they ordered from a vendor) to block CLK2 or activate Akt-mTORC1, dendritic spine levels were recovered. In addition, Andreas Lüthi’s group confirmed that neuronal electrical activity was restored. Here, the frequency of transmission events across synapses was rescued.

The team repeated the experiment with cells from patients with Phelan-McDermid syndrome. The researchers took skin cells from these patients, reprogrammed them into stem cells, and then coaxed them to become neurons. Again, the team was able to rescue synaptic transmission in the Shank3-deficient neurons by applying the tool compounds.

The final step was to show an impact on behavior. Enter Pico Caroni’s group. Like patients with autism spectrum disorders, Shank3-deficient mice have social deficits. Mice are typically social creatures, but Shank3-deficient mice tend to avoid their peers. When the team blocked CLK2 in Shank3-deficient mice, sociability was restored as measured by willingness to interact. Specifically, when given the option of retreating to a safe compartment or exploring a strange mouse, Shank3-deficient mice chose to be social following treatment with the tool compound.

“As a team, we were able to connect the dots between the molecular biology, the neural circuit and the behavior,” says Galimberti. “This represents a convincing preclinical package that justifies new drug discovery activities for Phelan-McDermid syndrome and autism.”

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