First proteomic analysis of birth defect demonstrates power of a new technique

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A UB team led by Fliesler revealed important information on a rare, sometimes deadly, birth defect. Credit: Sandra Kicman, University at Buffalo

The first proteomic analysis of an animal model of a rare, sometimes deadly birth defect, Smith-Lemli-Opitz Syndrome (SLOS), has revealed that the molecular mechanisms that cause it are more complex than previously understood. SLOS involves multiple neurosensory and cognitive abnormalities, mental and physical disabilities, including those

affecting vision and in severe cases, death before the age of 10.

The research, published by University at Buffalo scientists on Aug. 26 in *Molecular and Cellular Proteomics*, is the first to demonstrate a broad range of protein changes in the retina of a rat model of SLOS. To study SLOS, the UB researchers focused on the retina, which undergoes progressive degeneration as a result of SLOS. They compared protein expression in the retinas of rats with SLOS to those of healthy rats.

Since the 1990s, when it was discovered that SLOS involves defective cholesterol biosynthesis, much of the research on the disease has tended to emphasize only cholesterol metabolism, explains Steven J. Fliesler, PhD, senior author on the paper and Meyer H. Riwchun Endowed Chair Professor, vice chair and director of research in the UB Department of Ophthalmology and research health scientist at the Veterans Affairs Western New York Healthcare System.

"Only a few reports in the literature address the non-lipid constituents of cells and tissues in people affected with this disease," he says. "We had some clues that there were changes in gene expression in SLOS and since genes code for proteins, not lipids, we figured that maybe there are also significant proteomic changes involved.

"This is the first time anyone has looked at protein changes in this disease model and we found hundreds of them," continues Fliesler, who also is professor of biochemistry. While there are genetic mouse models of the disease, they have limited utility since they only live for one day while the retina (Fliesler's main area of interest) takes about a month after birth to form and fully mature in rodents.

"The SLOS rat model we used is able to live for at least three months, during which time the retina undergoes progressive degeneration," says Fliesler. He adds that while the retina in the SLOS animal model

degenerates, it is not yet known if the retina in humans initially undergoes normal development and subsequently degenerates in the course of the disease.

The UB research also provides the first glimpse of how cells in the retina die in this animal model, an observation that was provided by co-author Matthew Behringer, who conducted the research as a UB undergraduate in the Department of Biochemistry in the School of Medicine and Biomedical Sciences.

"Through this proteomic analysis, we found that the photoreceptor (rod and cone) cells die not through conventional programmed cell death (or apoptosis) but through some alternative mechanism, which is still under investigation," Fliesler explains.

To explore the proteomics of the SLOS rat model, the UB researchers, led by co-corresponding author Jun Qu, PhD, associate professor in the UB Department of Pharmaceutical Sciences in the School of Pharmacy and Pharmaceutical Sciences and the Department of Ophthalmology, used ion current based proteomic profiling, a relatively new and sophisticated methodology for studying proteins.

"This paper demonstrates that ion current based proteomic profiling is superior to conventional methods and could be broadly applicable to more common diseases, such as diabetes, cardiovascular disease, Alzheimer's Disease and age-related macular degeneration," says Fliesler.

Proteomic profiling is a method of studying differences in protein expression. Qu's lab is one of the national leaders in proteomic profiling on a large scale. The sophisticated methodology he and colleagues have developed was a key factor in the success of this research. The technique provides coverage for many more proteins than conventional techniques,

especially for numerous membrane-associated retina proteins.

Qu's work on this unique methodology eliminates a major source of false-positives that can occur in conventional proteomics analysis. Additional advantages of ion current based proteomic profiling are that it requires extremely small amounts of material, as little as 100 micrograms, and is objective, quantitative and highly reproducible. The method has been developed for a wide variety of biological specimens, ranging from microorganisms to humans.

The research exemplifies successful collaboration between two labs at UB that are part of the State University of New York Eye Institute, a SUNY-wide consortium funded by the SUNY REACH initiative, which brings together researchers in the ophthalmology departments of the four SUNY medical schools, including UB, as well as the SUNY College of Optometry and the College of Nanoscale Science and Technology.

"Thanks to the SUNY Eye Institute and SUNY REACH, we have a proteomics core module and we promote collaborations across the SUNY Eye Institute, which utilize this kind of methodology," explains Fliesler. "This facilitates our ability to do this kind of analysis in a very cost- effective manner within SUNY, as opposed to having to pay another institution or a private company for such analyses."

Provided by University at Buffalo