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Quantification of O-linked and N-linked glycome in human fibroblast

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Protein glycosylation is increasingly recognized as a crucial modulator of protein function, offering a third layer of biological information over genomics and proteomics. Modern tools for analyzing released N-glycans from cells, the glycome, have shown abnormal protein glycosylation in numerous human diseases. We developed a quantification of glycome in cells. Upon reaching 100% confluence, the cells were washed twice with PBS and harvested using a cell scraper. The cells were then pelleted and washed with PBS by centrifugation. Fibroblast pellets were lysed in more than 200 µl PBS, and 200 µg protein from the cell lysate was denature and precipitated with 2× volume of 100% propanol. N-linked and O-linked glycans were released from denatured protein, desalted and permethylated before subject to MALDI-TOF analysis. The quantification of O-linked glycans was achieved by spiking glycans from cell with 25 µM of C13-labelled T antigen (m/z 543) and C13-labelled sialylated T antigen (m/z 909). N-linked glycans were quantified using 25 µM of C13-labelled Man7GlcNAc2 (m/z 1107). Using this method, we identified abnormal fibroblast glycomes in a number of known patients with congenital disorders of glycosylation and demonstrate cellular glycome as an important tool for diagnosis of these diseases.

Biography

Xueli Li completed her PhD from Leipzig University, Germany. She is a Research Associate in The Michael J Palmieri Metabolic Laboratory, Children's Hospital of Philadelphia, Philadelphia, PA. She has more than 8 publications in the area of Glycomics.

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