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Cellular Glycomics – Recent Strategies and Approaches

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Cell surface glycans existing as glycolipids, glycoproteins and proteoglycans, known as "the cellular glycome", play fundamental biological functions and vary between cell types, stages of development and differentiation, and during disease development. Analysis of the cellular glycome provides a basis for understanding the functions of glycans in these cellular processes and molecular mechanisms of these cellular events as well. Recently, a variety of techniques have been developed to analyze the cellular glycome. These technologies include (i) lectin microarray, (ii) glycan microarray, (ii) MS spectrometry, and (iv) imaging cell surface glycans focusing on cellular glycomics, and are briefly highlighted below.

Lectins have been used as useful tools to characterize cell surface glycans due to their specific binding with glycans in specific linkage and configuration [1]. Lectin microarray has been developed as a useful tool for rapid profiling of glycans [2]. These microarrays were made by immobilization of lectin onto a solid support at a high spatial density. Interrogation of these arrays with fluorescently labeled samples creates a pattern of binding that depends on the carbohydrate structures, providing a method for the rapid characterization of carbohydrates. Lectin microarray was previously restricted to glycoproteins of cell lysates, recently, has been applied to analyze cellular glycomes directly. Hirabayashi et al. [3] reported lectin microarray technology for direct analysis of the live mammalian cell-surface glycome. In this study, fluorescent-labeled live cells were applied in situ to the established lectin microarray consisting of immobilized lectins with distinctive binding specificities. Bound cells on the array were directly detected by an evanescent-field fluorescence scanner in a liquid phase without fixing and permeabilization. Their results showed the capability of lectin microarray for differential profiling of CHO and its glycosylation-defective mutant cells, and splenocytes of wildtype and β1-3-N-acetylglucosaminyltransferase II knockout mice. They also compared cell surface glycans of K562 cells before and after differentiation and found a significant increase in the expression of *O*-glycans on differentiated cells. These results demonstrated that the technique provides useful strategy for profiling global changes of the mammalian cell surface glycome. In another study by Mahal et al. [4] a ratiometric two-color lectin microarray method was used to analysis of mammalian cell surface glycome. This study was inspired by the two-color ratiometric approach used in DNA microarray analysis. Briefly, cells are harvested by scraping or centrifugation. Then, the cell samples are sonicated to afford micellae-like structures (cellular micellae), which were isolated by means of ultracentrifugation. These cellular micellae are fluorescently labeled with orthogonal dyes (i.e., Cy3 or Cy5). Then, equivalent amounts of the appropriate Cy3- and Cy5-labeled samples are mixed and hybridized to each lectin microarray, and using known lectin specificities to interpret the array data into carbohydrate patterns. Overall, the reported lectin microarray techniques provide various advantages, such as (i) a complete set of cellular glycome can be analyzed in an intact state, (ii) experiments are carried out in a rapid and high-throughput manner, and (iii) cells remain viable throughout analysis. On the other hand, there are some major limitations of the live cell-targeted lectin microarray. First, absolute amounts of glycans cannot be determined directly from the signal intensities; this limitation is common to all other lectin microarray systems. Second, accurate whole glycan structures cannot be determined.

It is a challenge to characterize cellular glycolipid and identify their functions. Particularly, qualitative and quantitative techniques for the analysis of glycolipids are highly demanded. Glycosphingolipids (GSLs) are crucially important components of the cellular membrane. Shinohara et al. [5] reported a procedure for the elucidation of cellular GSL-glycomes based on (*a*) enzymatic glycan cleavage by endoglycosylceramidases in combination with (*b*) glycoblotting-assisted sample preparation. In this study, the mixture of endoglycosylceramidase I and II was employed to maximize the release of glycan moieties from the major classes of GSLs (i.e. ganglio-, (neo) lactoandglobo-series GSLs). The glycoblotting technique enabled the quantitative detection of GSL-glycans. Thirty-seven different kinds of cellular GSL glycans were successfully observed in 11 kinds of cells, including Chinese hamster ovary cells and their lectin-resistant mutants as well as murine and human embryonic carcinoma cells. Furthermore, MALDI-TOF/TOF mass spectrometry analysis and/or linkage-specific glycosidase digestion were used for in-depth structural clarification in terms of discrimination of isomers. This analytical technique was shown to be capable of delineating cell-specific GSL glycomes.

Although enzymatic release of the glycans from GSLs is feasible, the complete remove of the sphingosine moiety of GSLs may compromise the biological activity of GSLs regarding to their binding affinity and selectivity, particularly, GSLs comprise microdomains with many critical biological functions. To address these issues, Smith et al. [6] developed a strategy termed shotgun glycomics focusing on GSLs. They derivatized GSLs extracted from cells with a heterobifunctional fluorescent tag suitable for covalent immobilization as well. The fluorescent GSLs were separated by multidimensional chromatography, quantified and coupled onto glass slides to create GSLs shotgun microarrays for interrogation by Glycan Binding Proteins (GBPs) and antibodies. The reported shotgun glycomics incorporating GSLs is a strategy for focusing structural analyses on functionally important glycolipids and is expected to be approach for accessing the cellular glycomes of animal cells.

Cell surface glycans are targets for molecular imaging. In an emerging strategy, cell surface glycans are imaged by metabolic labeling with chemical reporters and subsequent ligation to fluorescent probes. This technique has enabled visualization of glycans in living cells and offers a new avenue for probing changes in cellular glycome that accompany development and disease. Bertozzi and Laughlin [7] have been pioneering the cellular sialic acid-containing glycans imaging by metabolic labeling with analogs of its biosynthetic precursor *N*-acetylmannosamine (ManNAc) or with derivatives of sialic acid that

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carry bioorthogonal groups (for example, azide, alkyne, ketone and aldehyde), that allows selective and biocompatible chemical ligation of reporter groups onto cell surface glycoconjugates. The key issue is that the biosynthetic machinery tolerates the addition of chemical reporters to the *N*-acyl group of either substrate class. For example, when mammalian cell lines are incubated with Ac4ManNAz, SiaNAz replaces 4–41% of natural sialic acids [8], which has been used to visualize sialic acids in a diverse range of cell types, as well as living mice and zebrafish. On the other hand, Hsieh-Wilson et al. [9] reported a chemoenzymatic strategy for detection of Fucα(1-2)Gal glycans, which is involved in many important physiological processes, such as learning and memory, inflammation, asthma, and tumorigenesis. They demonstrated that the approach is highly selective for the Fucα(1-2) Gal motif, detects a variety of complex glycans and glycoproteins, and can be used to profile the relative abundance of the motif on live cells, discriminating malignant from normal cells.

Aldehyde is a convenient functional group for chemical ligation since there are few native aldehydes found at the cell surface and can be selectively introduced into sialic acid containing glycoconjugates by periodates oxidation. Combination of periodate oxidation with aniline-catalyzed ligation (PAL) has become a viable method for detection of glycoconjugates on live cells. Paulson et al. [10] reported the PAL method, first introducing aldehydes onto cell surface sialic acids *via* mild peroidate oxidation for subsequent ligation with aminoxyl-biotin tag as for detection with fluorescein-labeled streptavidin. On the other hand, Ju et al. [11] reported a Chemiluminescent (CL) imaging method for in situ monitoring of cell surface glycan expression through chemoselective labeling of carbohydrate motifs by selective oxidization of of sialyl and galactosyl groups on cell surfaces into aldehydes by periodate and galactose oxidase, respectively, and then aniline-catalyzed hydrazone ligation with biotin hydrazide for specific recognition to avidin. This method was tested for distinguishing cancer cells from normal cells and monitoring of dynamic glycan expression on living cells. Most recently, Cairo et al. [12] reported two fluorescent nitrobenzoxadiazole dyes for labeling sialic acid moieties of glycoproteins and cell surface glycoconjugates using PAL and fluorescence spectroscopy. These carbonyl-reactive chromophores provide a one step alternative to avidin–biotin labeling strategies and simplify the detection of sialic acid in cells and glycoproteins. Molecular imaging enables visualization of specific molecules *in vivo* and without substantial perturbation to the target molecule's environment. However, there are some major limitations of the live cell-imaging. First, absolute amounts of glycans cannot be determined from the signal intensities. The reaction efficiency of metabolic engineering, enzymatic modification, or chemical modification and ligation is the key issue for quantitative evaluation of the cell surface glycans. Also, accurate complex glycan structures cannot be determined.

Overall, the cellular glycome varies at every level of biological organization, and in response to intrinsic and extrinsic stimuli. Glycan-binding proteins, pathogens and antibodies bind these glycans to mediate a wide diversity of biological events of either physiological or pathological pathway. Profiling the cellular glycome has been emerging as a very important research for both basic research and biomedical applications. This editorial highlighted recent cellular glycomic strategies and approaches, including, (i) lectin microarray, (ii) glycan microarray, (ii) MS spectrometry, and (iv) imaging cell surface glycans. These approaches provide potential tools for cellular glycomics, understanding the roles of this important class of carbohydrates in physiology and disease, and for exploring glycan-based biomarkers. Continued effort to overcome the drawbacks of the current techniques is needed. Particularly, approaches incorporating both quantitative and functional information about cellular glycans are highly demanded.

References

1. Sharon N, Lis H (2004) History of lectins: from hemagglutinins to biological recognition molecules. Glycobiology 14: 53R-62R.
2. Pilobello KT, Slawek DE, Mahal LK (2007) A ratiometric lectin microarray approach to analysis of the dynamic mammalian glycome. Proc Natl Acad Sci U S A 104: 11534-11539.
3. Hirabayashi J, Tateno H, Uchiyama N, Kuno A, Togayachi A, et al. (2007) A novel strategy for mammalian cell surface glycome profiling using lectin microarray. Glycobiology 17: 1138-1146.
4. Mahal LK, Hsu KL, Pilobello K, Krishnamoorthy L (2011) Ratiometric lectin microarray analysis of the mammalian cell surface glycome. Methods Mol Biol 671: 117-131.
5. Shinohara Y, Fujitani N, Takegawa Y, Ishibashi Y, Araki K, et al. (2011) Qualitative and quantitative cellular glycomics of glycosphingolipids based on rhodococcal endoglycosylceramidase-assisted glycan cleavage, glycoblotting-assisted sample preparation, and matrix-assisted laser desorption ionization tandem time-of-flight mass spectrometry analysis. J Biol Chem 286: 41669-41679.
6. Smith DF, Song X, Lasanajak Y, Xia B, Heimburg-Molinaro J, et al. (2011) Shotgun glycomics: a microarray strategy for functional glycomics. Nat Methods 8: 85-91.
7. Bertozzi CR, Laughlin ST (2009) Imaging the glycome. Proc Natl Acad Sci U S A 106: 12-17.
8. Luchansky SJ, Argade S, Hayes BK, Bertozzi CR (2004) Metabolic functionalization of recombinant glycoproteins. Biochemistry 43: 12358-12366.
9. Hsieh-Wilson LC, Chaubard JL, Krishnamurthy C, Yi W, Smith DF (2012) Chemoenzymatic probes for detecting and imaging fucose-α(1-2)-galactose glycan biomarkers. J Am Chem Soc 134: 4489-4492.
10. Paulson JC, Zeng Y, Ramya TN, Dirksen A, Dawson PE (2009) High-efficiency labeling of sialylated glycoproteins on living cells. Nat Methods 6: 207-209.
11. Ju H, Han E, Ding L, Qian R, Bao L (2012) Sensitive chemiluminescent imaging for chemoselective analysis of glycan expression on living cells using a multifunctional nanoprobe. Anal Chem 84: 1452-1458.
12. Cairo CW, Key JA, Li C (2012) Detection of cellular sialic acid content using nitrobenzoxadiazole carbonyl-reactive chromophores. Bioconjugate Chem 23: 363-371.