Glycan Profiling of Living Cell Surface

Measurement of glycome on the cell without breakage

GlycoStation™ 3

Technical Information

Glycotechnica

Glycome Profiling of CHO and Lec1 Mutant cell lines

The entire of glycans of an organism is called "Glycome" in a similar manner of genome and proteome. It is well known that the cell surface glycome changes by species, differentiation stages, disease conditions, and mutations.

In the case of GlycoStation™, the standard protocol is based on fluorescent labeling onto the protein part (actually to the amino-group) of glycoproteins. However, at a recent research, it was show that the measurement of glycome on living cell surface is possible by a different labelings method using a Cell-Tracker Orange CMRA reagent, which is metabolically converted to fluorescent derivative inside the cell . One of the advantages of this method is that it becomes possible to measure not only glycoproteins but also glycolipids on cell surfaces. (Fig.1)

In this thechnical note, we show the comparison of glycome on CHO cells with that on Lec1 mutant cells, from which GlcNAc-transferase Ⅰ was knocked out, using lectin microarray. With using CMRA reagents into these two kinds of cells, the differences in the glycomes are clearly detected from the fluorescent patterns as a result of interaction between glycans of living cells and lectins immobilized on LecChip™.

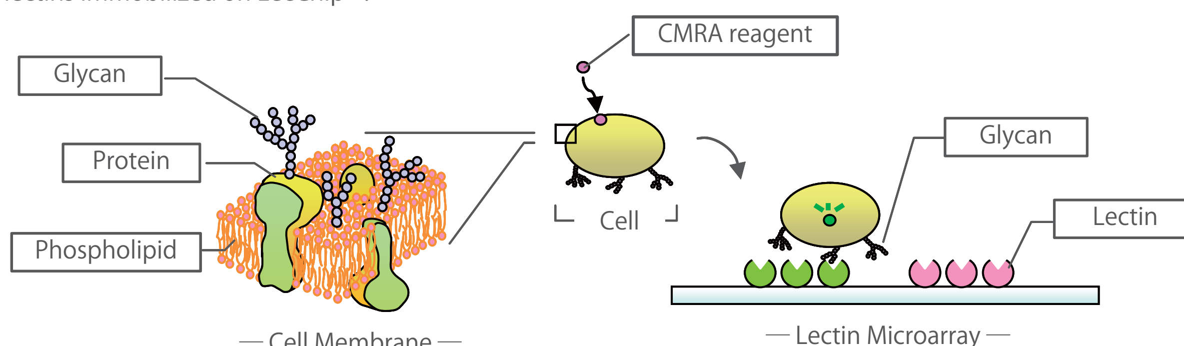

Fig. 1 Living cell glycome profiling

◆ Fluorescent mesurement images and microscopic image

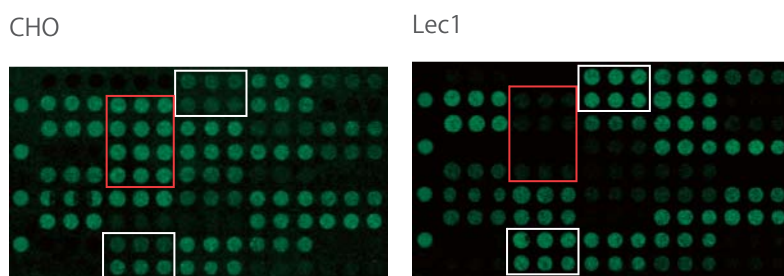

Fig. 2 Fluorescent measurement image

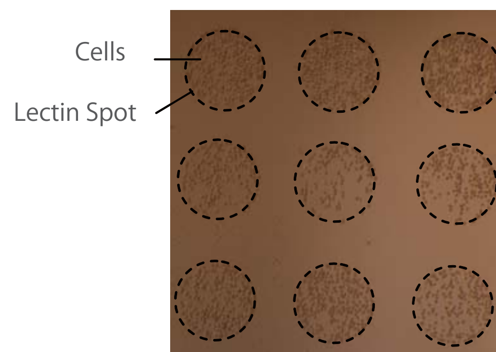

Fig. 3 Micrograph

GlcNAc-transferase Ⅰ plays important role in adding GlcNAc onto the α1-3 and α1-6 Man of complex and hybrid type N-glycan core structure. Therefore, in the case of GlcNAc-T Ⅰ deficient Lec1 mutant, we can theoretically expect that Lec1 lacks complex and hybrid type N-glycans, resulting in increase of high-mannose type N-glycans instead.

Actually, we could observe clear difference between the two fluorescent images(Fig.2). Moreover, the appearance of cells binding on the lectin spots can be observed with a microscope, because the cells are still alive. (Fig.3)

◆ Characteristic differences in lectin signals

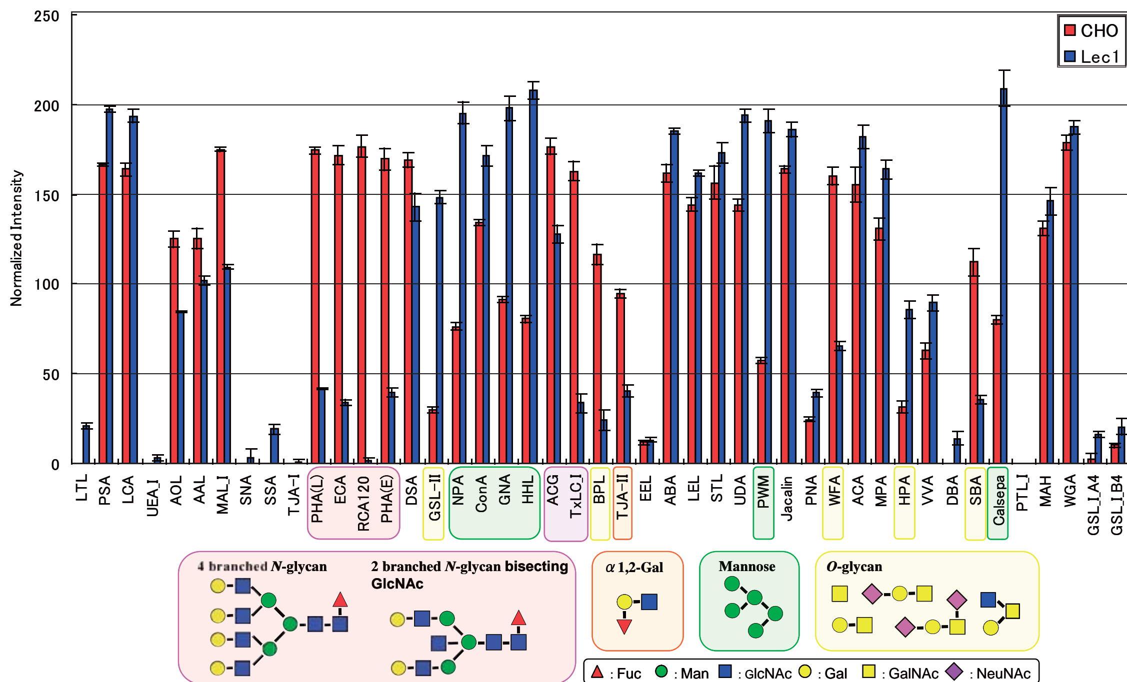

Fig.4 Signal comparison between CHO and Lec1

We observed characteristic differences in N-glycan binding lectins with the lectin microarray analyses. The signals for complex type N-glycan binders (PHA(L), PHA(E), ACG, TxLC Ⅰ) and that for asialo binders (ECA, RCA120) decreased in Lec1, whereas the signals for high-mannose binders (NPA, ConA, GNA, HHL, PWM, Calsepa) increased in Lec1. It is considered that the differences in signals for O-glycan binders are due to the fact O-glycans and/or glycolipids might be spatially more exposed at the surface of Lec1 mutant cells as a result of the absence of much larger complex-type N-glycans.
These findings coinside with the fact that Lec1 is a GlcNAc-TI deficient mutant reasonably. (Fig.4)

- Protocol -

1. Collection of cell sample

1-1. Retrieve 3×10^6 cells[1] and wash those with serum-free culture media.

2. Fluorescent labeling

2-1. Add 3ml CellTracker™ [2] to the cells that were suspended with serum-free culture media.
2-2. Incubate the sample for 15min at 37℃ stirring under dark condition.
2-3. Confirm the cell has been dyed by washing the cells with 1%BSA/PBS after the incubation.

3. Measurement

3-1. Dilute the dyed cells with 1%BSA/PBS.
3-2. Wash a LecChip™ [3] three times with Probing Solution[4], then add samples into wells（100ml / well）.
3-3. Incubate the LecChip for 30min at 4℃.
3-4. Put the incubated LecChip into the 50ml tube that filled with PBS, then stand for 30min with the well-side below. The cells that doesn't bind with the lectin are removed by gravity.
3-5. Scan the LecChip with GlycoStation™ Reader 1200[5].
3-6. Analyze the results with Array-Pro™ Analyzer[6] and GlycoStation™ Tools[7].

Note

1) Use cells which are passaged once at least.
2) CellTracker™ Orange CMRA (Invitrogen, Cat#:C34551) Make to 1mM adding DMSO. Use to final waking concentration of 10mM.
3) LecChip™ (Glycotechnica)
4) Probing Solution (Glycotechnica)
5) GlycoStation™ Reader 1200 (Glycotechnica)
6) Array-Pro™ Analyzer ver.4.5 (MEDIA CYBERNETICS)
7) GlycoStation™ Tools (Glycotechnica)

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