Glycan Profiling of Crude Sample

Comparison of CHO and Lec1 mutant cell lines

As is well know, cells have specific glycomes depending on the origins: species, organs, differentiation stages, and/or helth conditions. Therefore, glycan are usually called “the face of the cell”. Up until the present, there have not been any simple and high-sensitive ways to identify cells focusing on the difference of glycomes.
In this technical note, we compare glycomes between CHO and Lec1 mutant cells, from which GlcNAc-transferase I was knocked out, using GlycoStation™. The differences in glycomes are detected from fluorescence patterns due to interactions of Cy3 labeled cell membrane glycoproteins with lectins immobilized on LecChip™.

◆ GlcNAc-transferase I

As is shown in Fig.1, GlcNAc-transferase I (GlcNAc-T 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 a 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.

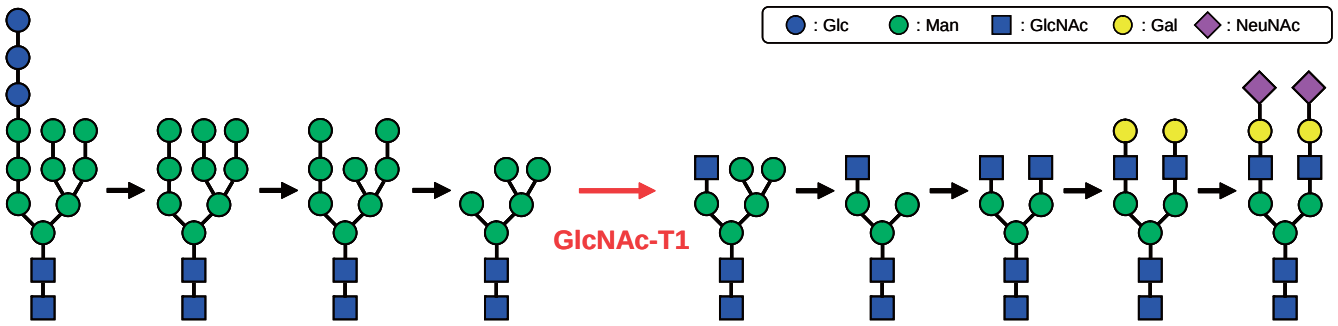

Fig.1 GlcNAc-T I works to change glycan structures from high-mannose to complex and hybrid type N-glycans.

◆ Characteristic differences in lectin signals

We observed characteristic differences in N-glycan binding lectins from the lectin microarray analyses. The signals of complex type N-glycan binders (PHA(L), PHA(E), ACG), that for α2,3-Sia binder (MAL I), and that for asialo binders (RCA120) decreased in Lec1 (see Fig.2(A)), whereas the signals of high-mannose binders (GNA, HHL, PWM, Calsepa, PSA, LCA) increased in Lec1 (see Fig.2(B)). These findings are quite reasonable taking into consideration that Lec1 is a GlcNAc-T I deficient mutant.

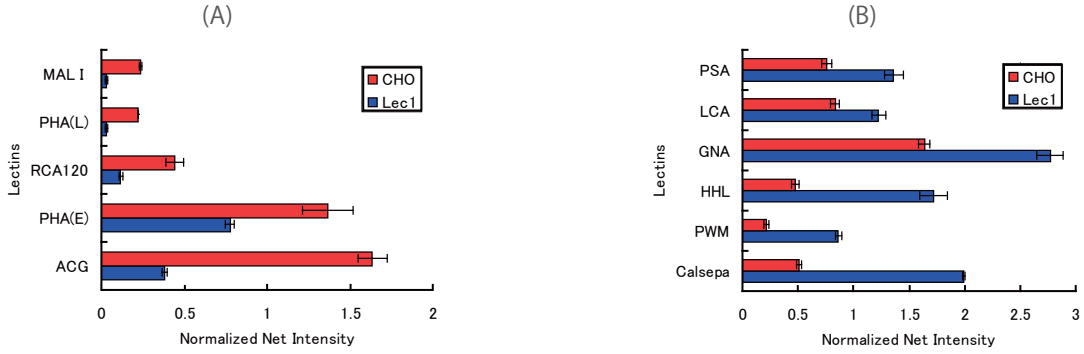

Fig. 2 Comparison of Cy3 fluorescence intensities between CHO and Lec1 mutant cells
(A) Lectin signals higher in CHO (B) Lectin signals higher in Lec1

◆ Dependence of signal intensity on sample concentration

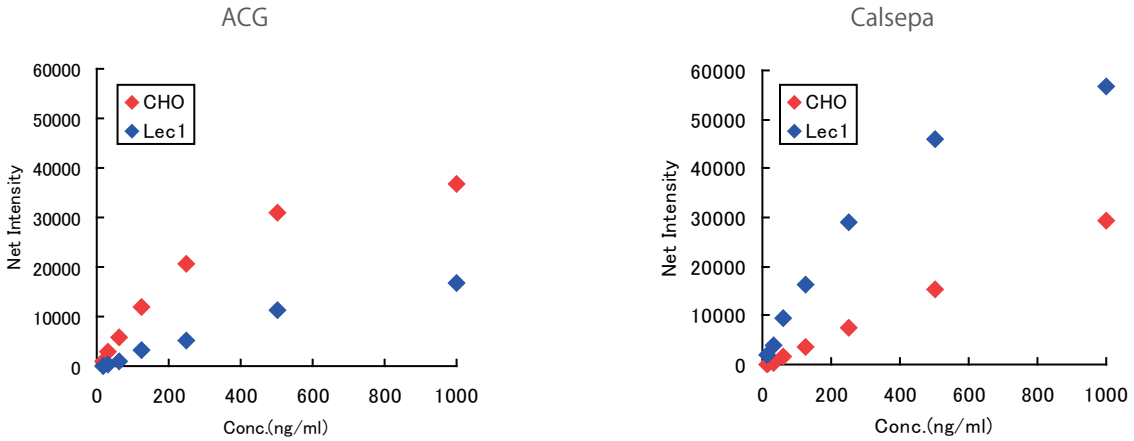

Fig.3 Concentration dependent signal intensity curves for ACG and Calsepa

Quantitative differential comparison is possible in GlycoStation™, utilizing a fact that the lectin microarrays signals get strnger with increasing sample concentration. Fig.3 shows concentration dependent signal intensity curves for ACG and Calsepa using membrane fractions taken from CHO and Lec1 mutant cells. For your information, ACG typically binds to Sia α2-3Gal β1-4GlcNAc, and Calsepa has specificity to monnose type N-glycans. These findings promise you that the result of differential analysis doesn't change with sample concentration and the profiling pattern is also kept constant, as far as the experiment is done in a proper concentration range without singal saturation. This is one of the great appealing points of GlycoStation™.

- Protocol -

1. Sample preparation and fractionation

1-1. Wash cells (5×10^6) by PBA several times and store at -80℃.
1-2. Fractionate the cells into membrane and cytoplasm fractions by using a commercial Kit[1].

2. Cy3 labeling

2-1. Measure protein concentration with a commercial Micro BCA Protein Assay Reagent Kit[2] (reaction time = 2h).
2-2. Prepare 20μL sample volume with a concentration of 50μg/ml using PBS[3], then mix it with Cy3 Mono-Reactive dye 100μg labeling[4] .
2-3. React it for 1h in a dark place at R.T.
2-4. Add 300μL TBS[6] into a gel filtration column[5], then centrifuge at 1,500×g, for 1min at 4℃.
2-5. Repeat it two times.
2-6. Add the whole sample prepared at 2-3 and 25μL TBS into the gel filtration column prepared at 2-5, then centrifuge at 1,500×g, for 2min, at 4℃, and remove excess Cy3.

3. Measurement

3-1. Dilute samples with a Probing Solution[7] (in a range from 2μg/mL to 31.25ng/mL).
3-2. Wash LecChip™ [8] by a Probing Solution three times、then apply samples into wells (100μL/well).
3-3. Incuvate LecChips at 20℃ over night.
3-4. Measure fluorescence patterns without any washing of LecChips by GlycoStation™ Reader 1200[9].
3-5. Analyse the results by Array-Pro™ Analyzer[10] and GlycoStation™ Tools[11].

Note

1) ProteoExtract® Subcellular Proteome Extraction Kit (Calbiochem, #539790)
2) Micro BCA Protein Assay Reagent Kit (PIERCE, #23235)
3) PBS(-) pH7.3
4) Use the equivalent amount of Cy3 Mono-Reactive dye pack (GE, #PA23011) to that used for 100μg protein labeling.
5) Zeba Desalt Spin Columns, 0.5ml (Thermo,#89883)
6) TBS pH7.5
7) Probing Solution (GlycoTechnica)
8) LecChip™ (GlycoTechnica)
9) GlycoStation™ Reader 1200 (GlycoTechnica)
10) Array-Pro™ Analyzer ver.4.5 (MEDIA CYBERNETICS)
11) GlycoStation™ Tools (GlycoTechnica)

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