# Glycan Profiling of Secreted Proteins from Cells

## By using cultivated cancer cells

GlycoStation™ 2

Technical Information

Glyco Technica

### Comparison of normal hepatic cell (NKNT-3) with the cancerous cell (NKNT-3/3-9-2)

Almost all of secreted proteins are glycosylated, and it is well known that eventual glycan structures are different from organ to organ and also change with helth conditons. For instance, it is also well know that existing tumor markers like CA19-9 and AFP-L3 are based on detecting change of glycan structures.

In this technical note, we will discribe comparison of normal hepatic cell (NKNT-3), which was immortalized by SV40 large T-antigen, with the cancerous cell (NKNT-3/3-9-2), with using secreted glycoproteins into culture media.

### ◆ Fluorescense patterns taken at the same protein concentration

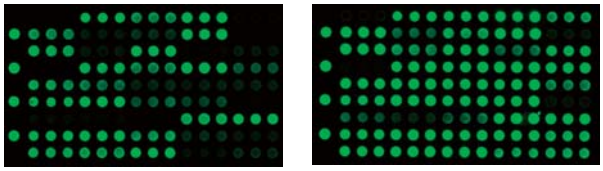
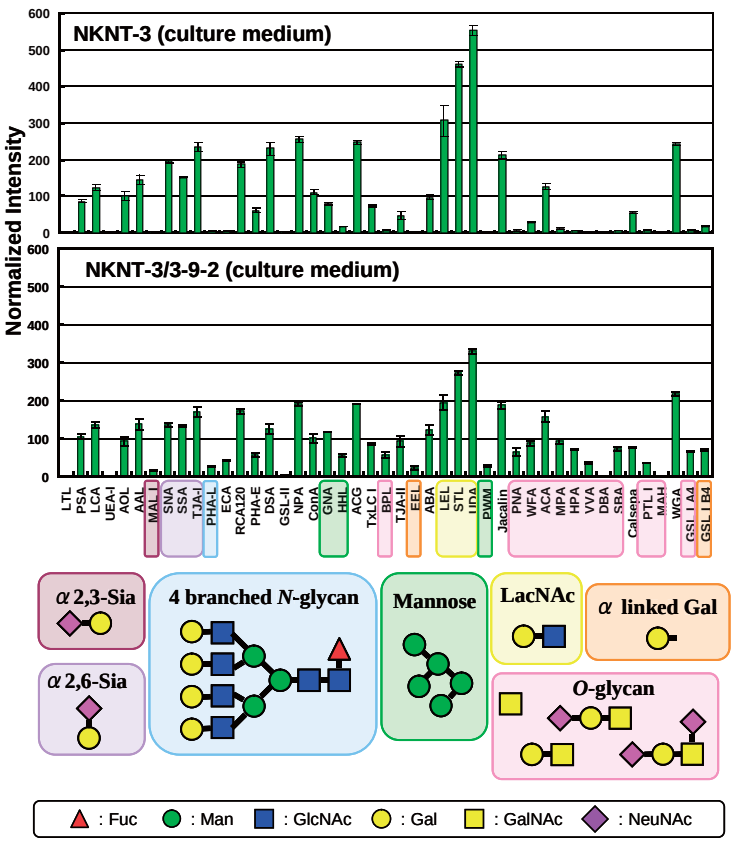


Fig. 1 Fluorescence patterns of culture supernatants of NKNT-3 and NKNT-3/3-9-2

First of all, we compared fluorescence patterns between NKNT-3 and NKNT-3-9-2 at the same protein concentration. We have found suprizingly that the overall lectin signals from 45 different lectins get significantly higher in NKNT-3/3-9-2 than those of NKNT-3. We believe this fact suggests that the expression level of glycans are higher in cancer cells than normal ones (shown in Fig. 1).

### ◆ Glycan profiling patterns




Then, we analyzed differences in lectin signal intensities taken by LecChips in detail. In this case, we normalized glycan profiling patters over 45 different lectins by setting the average of 45 lectin intensities to 100 (shown in Fig.2). You can clearly understand that the glycan profiling patters are totally different between normal hepatic cell (NKNT-3) and the cancerous cell (NKNT-3/3-9-2). For instance, α2,3-Sia binder (MAL I ), four branched complex type N-glycan binder (PHA(L)), α-Gal binders (EEL, GSL I B4), high-mannose binders (GNA, HHL, PWM), O-glycan binders (BPL, PNA, WFA, MPA, HPA, VVA, SBA, PTL I , MAH, GSL I A4) showed higher signal intensities in NKNT-3/3-9-2. On the other hand, you can also find that N-acetyl Lactosamine binders (LEL, STL, UDA), α2-6 Sia binders (SNA, TJA I ) showed weaker signal intensities in NKNT-3/3-9-2.

Thus, we could easily find changes in glycan structures with developing cancers.

Fig. 2 Glycan differential profiling and estimated difference in glycan structures

This result is based on the collaboration with Prof. Nakabayasi of Hokkaido Info. Univ.

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## Glycan profiling for several human tumor cell lines

We compared cyclopaedically glycan profiling patterns taken from other tumor-derived cell lines such as hepatoblastoma, yolk sac tumor, stomach cancer, breast cancer, and so on, with using the same technology as human hepatic cancerous cells.

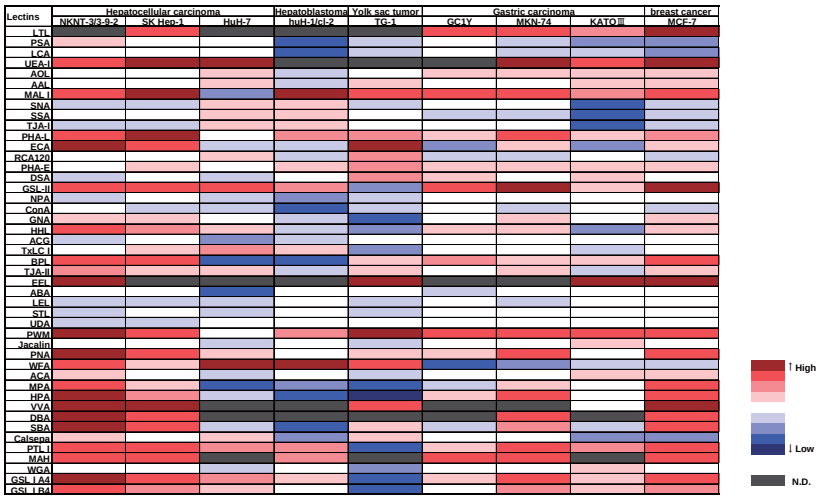


Fig. 3 Clear differences in various glycan expression levels among several human tumor cell lines

Fig.3 shows comparative display of lectin signal intensities with up-regulated one in red and down-regulated one in blue using NKNT-3 as the control.

It is clearly shown that each cell line has specific glycan profiling pattern as is expected.

As is often said that glycans are "the face of the cell", we could show the same idea even from secreted proteins that those represent clearly the nature of their origins, and therefore could be used to identify disease states using a fingerprint like identification strategy.

## - Protocol -

### 1. Enrichment of secreted proteins and buffer exchange

1-1. Collect 10mL[1)] of culture supernatant and store it at -80℃.

1-2. Melt the sample 1-1, and apply it to a barrier filter[2)]. Centrifuge it at 4,000×g, at 4℃, and thereby enrich the sample less than 500μL.

1-3. Add 14.5mL of PBS[3)] to the 1-2 result. Centrifuge it at 4,000×g, at 4℃, and enrich the samples less than 500μL (enrichment and buffer exchange).

1-4. Repeat the 1-3 process.

1-5. Add 14.5mL of PBS to the 1-4 result. Centrifuge it at 4,000×g, at 4℃, enrich the sample less than 250μL, and recover the sample.

1-6. Prepare 500μL sample adding PBS to the 1-5 result.

### 2. Cy3 labeling

2-1. measure protein concentration with a commercial Micro BCA Protein Assay Reagent Kit[4)] (reaction time = 2h).

2-2. prepare 20μL sample volume with a concentration of 50μg/ml using PBS, then mix it with Cy3 Mono-Reactive dye 100μg labeling[5)].

2-3. React it for 1h in a dark place at R.T.

2-4. Add 300μL TBS[6)] into a gel filtration column[7)], 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[8)] (in a range from 2μg/mL to 31.25ng/mL) .

3-2. Wash LecChip™ [9)] 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[10)].

3-5. Analyse the results by Array-Pro™ Analyzer[11)] and GlycoStation™ Tools[12)].

### Note

1) The culture supernatant used in this experment must be that of serum-free culture medium, or the culture medium must be replace serum-free one before collecting it.

2) Amicon Ultra-15, Ultracel-5K(MilliPore, #UFC900596)

3) PBS(-) pH7.3

4) Micro BCA Protein Assay Reagent Kit (PIERCE, #23235)

5) Use the equivalent amount of Cy3 Mono-Reactive dye pack (GE, #PA23011) to that used for 100μg protein labeling.

6) TBS pH7.5

7) Zeba Desalt Spin Columns, 0.5ml (Thermo,#89883)

8) Probing Solution (GP Biosciences)

9) LecChip™ (GP Biosciences)

10) GlycoStation™ Reader 1200 (GP Biosciences)

11) Array-Pro™ Analyzer ver.4.5(MEDIA CYBERNETICS)

12) GlycoStation™ Tools (GP Biosciences)

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