



GLYCOANALYSIS OF CELL MEMBRANE PROTEINS ON A LECTIN ARRAY

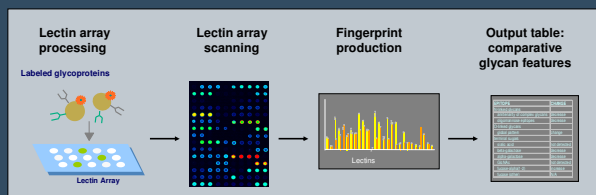
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ABSTRACT

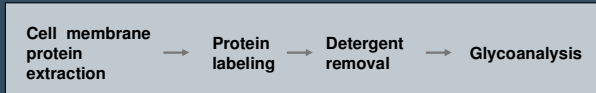
Protein glycosylation plays important structural and functional roles in many biological processes. Hence, exploring glycan structures on proteins is a major interest for understanding these processes in cells and body fluids. Procognia has developed a lectin-array based technology for analysing the glycan composition of glycoproteins. The technology does not require lengthy sample preparation, nor high analytical laboratory skills as with chromatographic and MS- based methods. The first product developed from this technology is a platform for glycoanalysis of therapeutic proteins, the GlycoScope. This product provides accurate, quantitative glycoanalysis for single proteins.

Based on this technology a kit for comparative glycoanalysis of membrane protein extracts from cultured cells was developed. The kit enables the assessment of differences in glycosylation patterns of membrane proteins from two comparable cell populations such as before and after drug treatment, before and after differentiation, metastatic and non-metastatic cancer cells and others. The kit contains a knowledge-based algorithm that compares the lectin binding fingerprints of the two populations to produce a list of changes in glycan epitopes (for example an increase in sialic acid). The applicability of this technology is demonstrated using various biological systems such as cells treated with the well characterized glycosylation inhibitor- 1-deoxymannojirimycin and cells exposed to the ER stress inducing agent, Brefeldin A, reported to affect cellular protein glycosylation.

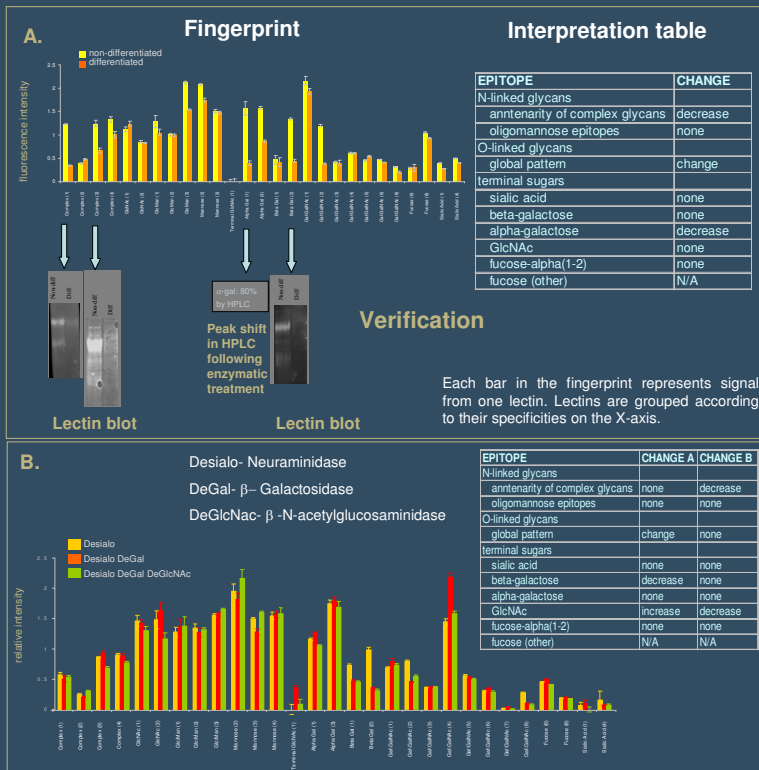
GLYCOANALYSIS WORKFLOW



SAMPLE PREPARATION



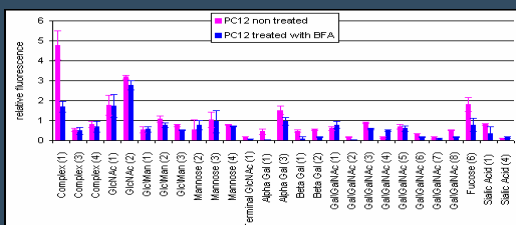
PROOF OF CONCEPT FOR GLYCOANALYSIS OF CELL MEMBRANE PROTEINS



APPLICATIONS IN RESEARCH AND DRUG DEVELOPMENT

Drug- Brefeldin A

Brefeldin A (BFA) is a known inducer of endoplasmic reticulum (ER) stress, leading to apoptosis. BFA inhibits protein transport from the ER to the Golgi apparatus, and has been shown to inhibit terminal glycosylation of complex N-linked glycans. BFA appears to fuse the ER and the Golgi compartments, but not the trans Golgi network (TGN). Therefore, the initial steps in the complex N-linked glycan synthesis, which occur in the cis- and medial Golgi, are inhibited only moderately. The later steps in N-glycan synthesis, like addition of the galactose, sialic acid and fucose, are performed in the TGN, and are therefore significantly inhibited by BFA (Sampath et al. (1992) J.Biol.Chem. 267, 4440-4455).



PC12 cells were treated with 12ug/ml BFA for 24h, harvested and analyzed on the lectin array

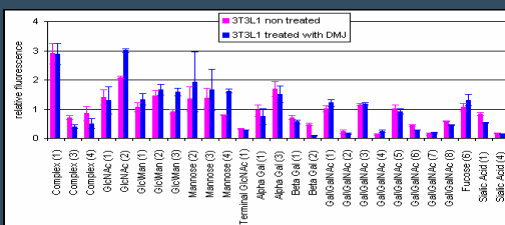
EPITOPE	CHANGE
N-linked glycans	
antennarity of complex glycans	decrease
oligomannose epitopes	none
O-linked glycans	
global pattern	none
terminal sugars	
sialic acid	none
beta-galactose	none
alpha-galactose	none
GlcNAc	none
lucose-alpha(1-2)	decrease
lucose (other)	N/A

Following BFA treatment a significant decrease in the antennarity of the membrane glycoproteins was detected by the interpretation software, accompanied by a decrease in antennary fucose (see table).

The fingerprint also demonstrated the expected decrease in signals from lectins recognizing antennae-termini such as alpha-galactose, beta-galactose and sialic acid.

N- Glycosylation inhibitor- DMJ

1-deoxymannojirimycin (DMJ), a known inhibitor of mannosidase I. The initial steps in N-glycan synthesis involves synthesis of a precursor oligosaccharide, which is then stepwise processed by several enzymes, including mannosidase I, to allow synthesis of complex N-linked glycans. DMJ blocks mannosidase I and therefore inhibits conversion of high mannose to complex chains. As a result, treatment with DMJ leads to synthesis of glycoproteins with increased levels of high mannose glycans and less complex N-linked glycans.



3T3L1 cells were treated with 0.8mg/ml DMJ for 3 days, harvested and analyzed on the lectin arrays

EPITOPE	CHANGE
N-linked glycans	
antennarity of complex glycans	decrease
oligomannose epitopes	increase
O-linked glycans	
global pattern	none
terminal sugars	
sialic acid	none
beta-galactose	none
alpha-galactose	none
GlcNAc	none
lucose-alpha(1-2)	none
lucose (other)	N/A

Following DMJ treatment a significant decrease in the antennarity of the membrane glycoproteins was detected by the interpretation software, accompanied by an increase in oligomannose (see table).

The fingerprint also demonstrated the expected decrease in signals from lectins recognizing antennae-termini such as alpha-galactose, beta-galactose and sialic acid.

LECTIN ARRAY GLYCOANALYSIS

simple	straightforward technology no need for special equipment simple material preparation low hands-on time
fast	12 samples in 3-5 hours by single researcher
applications	purified proteins proteins in complex mixtures global glycosylation patterns

SUMMARY

Kit was used to analyze changes in membrane proteins glycosylation from cells treated with:

- BFA
 - Expected decrease in antennarity and terminal sugars of glycoproteins was demonstrated.
- DMJ
 - Expected decrease in antennarity and increase in high mannose structures of glycoproteins were demonstrated.

The kit will be marketed by Qiagen in 2008