Glycosylation profile of cancer cells

OBJECTIVE

Determine the glycosylation profile of cancer cells using a cell based QCM Biosensor assay.

CONCLUSIONS

– A single surface can be used to perform lectin screening and to provide a highly relevant and reproducible determination of the glyco-profile of cancer cells (A-431)

– Real-time measurements provide a highly accurate quantitative and qualitative evaluation of lectin-cell interactions.

– The use of inhibitors may help to further characterize the composition of the glycotopes involved in the lectin binding.

BACKGROUND

In today's search for new cancer therapeutics and diagnostic tools, glycobiology has become a new focus. Changes in the glycosylation pattern of cancer cells as detected by carbohydrate binding proteins (lectins) have been reported to play a crucial role in cancer formation and in metastasis. However, there is a need for highly informative data to define both the nature of such glycans as well as their involvement in the cancer development process. The Attana Cell™ 200 makes global glycan profiling possible by using lectins of different specificities. A more accurate characterization may be achieved using a competition assay.

ATTANA CELL™ 200 BIOSENSOR

The Attana Cell 200 is a dual channel, label-free, temperature controlled, continuous flow system for manual (Attana Cell 200) or automated (Attana Cell A200) analysis of molecular interactions with cells.

The Attana Cell 200 system is characterized by the ability to study molecular interactions with cells grown directly on the sensor surface. Even higher biological relevance is achieved through features such as continuous flow, physiological temperatures and label-free detection. High data quality is achieved by direct measurements in real-time, avoiding disturbances caused by secondary detection.

The QCM core technology enables the study of biomolecules of varying species such as proteins, nucleic acids, carbohydrates, lipids and lectins and also binding moieties of vastly different sizes, ranging from peptides to cells.

METHOD

Preparation of cell sensor chips: A-431 and MDA-MB-468 cells were seeded onto the Attana MPT-1 cell sensor surface to a density of about 40000 cells per sensing area and placed at 37°C with 5% CO_2 in DMEM/glutamax medium supplemented with 10% fetal bovine serum. Following an incubation time of 24h, the cells were rinsed in PBS and fixed in 3.7% formaldehyde. The cell sensor chip was subsequently inserted into the Attana Cell 200 biosensor and stabilized overnight in running buffer (PBS pH 7.4 supplemented with 0.025% Tween® 20) at a flow rate of 25 µl/min.

Lectin screening: The following lectins (table 1) bearing various carbohydrate specificities were successively injected over the previously prepared MPT-1 cell sensor chip. Typically a lectin was injected at a concentration of 50 µg/ml (prepared in running buffer) with the association and dissociation phases being recorded using the Attester Software. The subsequent injection of lectin was performed after the surface had been regenerated using glycin 10 mM pH 1.0, supplemented with 0.5 M NaCl.

Competition assay: The binding of the lectin Con A to A-431 cells previously immobilized was monitored in presence of increasing concentration of various specific (mannose/glucose) or irrelevant (galactose) sugars. Con A was preincubated for 15 min in presence of the carbohydrate of interest and the mixture was subsequently flowed over the cell sensor. The resulting interaction was monitored and appropriately referenced. Maximal frequency shifts, recorded at the end of the injection phase, were used to plot inhibition isotherms and determine the inhibition potency of competing carbohydrates as well as to characterize the cell glycan structures.

Lectin name	Abbreviation	Specificity
Concanavalin A	Con A	Mannose/Glucose
Dolichos biflorus agglutinin	DBA	Alpha GalNAc
Peanut agglutinin	PNA	Galactose/T antigen
Soybean agglutinin	SBA	Alpha/beta GalNAc-Galactose
Ulex europaeus agglutinin-I	UEA-I	Fucose

Table 1: Lectin names and respective specificity (as provided by manufacturer)

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RESULTS

Lectin screening: Con A, DBA, PNA, SBA and UEA-1 at a concentration of 50 µg/ml were injected over the sensor surface with immobilized A-431 or MDA-MB-468 cells, and the binding curves were monitored using the Attester Software. The maximal frequency shift monitored at the end of the injection phase is depicted in Figure 1. A surface prepared under the same conditions but without cells was used as a control. The varying lectin binding observed highlighted major differences in the glycan composition of the two above-mentioned cancer cell types. The real time evaluation of the lectin binding using the QCM biosensor may enhance the accuracy of the measurements as compared to more classic approaches such as ELISA. Indeed, in the case of fast dissociating interactions such as those shown in Figure 2, the extent of the binding may be underestimated due to late measurements occurring after long washing steps in a cell-based ELISA assay, whereas more accurate data will result from using a biosensor cell-based assay measuring in real-time.

Competition assay: In order to characterize the glycotopes recognised by a lectin, a competition assay may be used as schematically depicted in Figure 3. As an example, the inhibition potencies of galactose, mannose and glucose are shown in the competition isotherm in Figure 4.

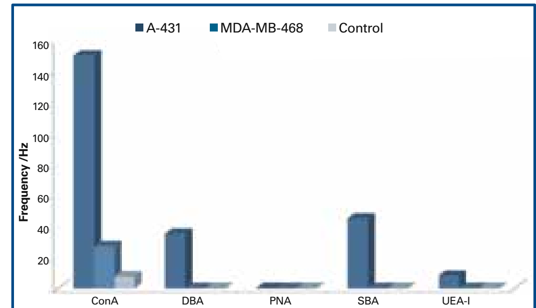

Figure 1. Lectin screening. The binding of Con A, DBA, PNA, SBA and UEA-I was quantified at the end of the association phase on three different surfaces; A-431 MDA-MB-468 and a control surface without cells.

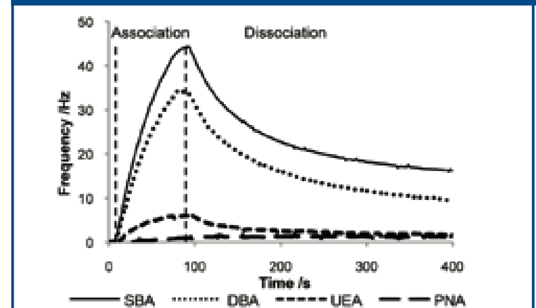

Figure 2: Binding profile of SBA, DBA, UEA-I and PNA as measured using the Attana Cell 200.

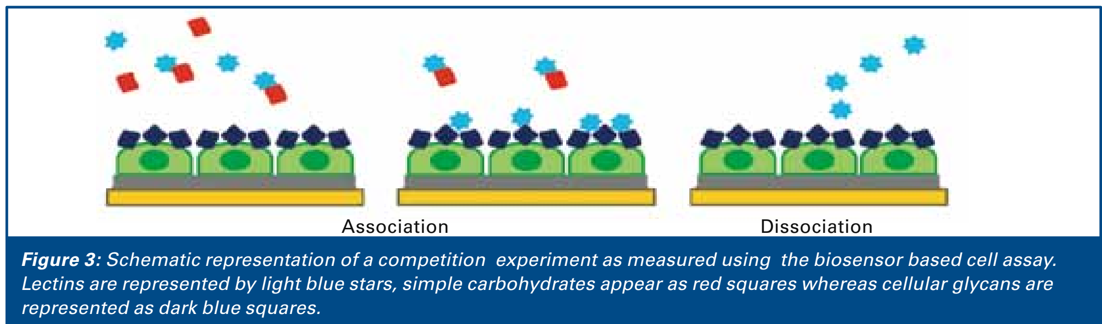

Figure 3: Schematic representation of a competition experiment as measured using the biosensor based cell assay. Lectins are represented by light blue stars, simple carbohydrates appear as red squares whereas cellular glycans are represented as dark blue squares.

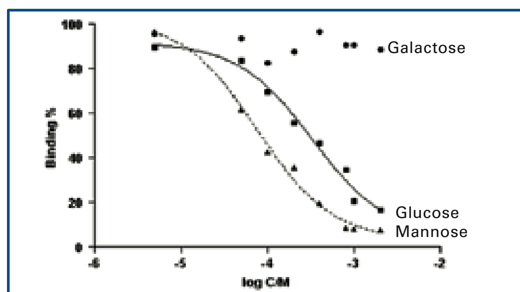

Figure 4: Competition assay. The interaction of the lectin Con A with A-431 cells was monitored in presence of mannose (dashed line), glucose (solid line) and galactose (dark dots).

Attana Materials	Item Code
Attana Cell™ 200	3746-3001
Attana MPT-1 Cell sensor chip	3621-3103
Attaché 2.0 Software suite	3470-3001

Attana AB
Björnnäsv. 21. SE-114 19 Stockholm, Sweden
Telephone: +46 8 410 200 00, E-mail: info@attana.com
www.attana.com

attana sensor technologies

www.attana.com