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A novel approach to determining the affinity of protein–carbohydrate interactions employing adherent cancer cells grown on a biosensor surface

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ABSTRACT

The development of biological agents for the treatment of solid tumours is an area of considerable activity. We are pursuing carbohydrate-binding proteins (lectins) in a strategy aimed at targeting cancer-associated changes in glycosylation. To evaluate lectin–cancer cell interactions we developed a novel cell biosensor in which binding events take place at the cell surface, more closely mimicking an *in vivo* system. Metastatic, SW620, and non-metastatic, SW480, colorectal cancer cells were grown on the surface of a tissue-culture compatible polystyrene coated biosensor chip and housed in a quartz crystal microbalance (QCM) apparatus, the kinetics of binding of a diverse range of lectins was evaluated. The lectin *Helix pomatia* agglutinin (HPA) has been shown to bind aggressive metastatic cancer and was produced in recombinant form (His- and RFP-tagged). The affinity of HPA was in the nanomolar range to the metastatic SW620 cells but was only in the micromolar range to the non-metastatic SW480. Overall, the dissociation constant (K_D) of the lectins tested in the new cell biosensor system was an order of magnitude lower (nanomolar range) than has generally been reported with systems such as QCM/SPR. This new cell-biosensor enables molecular interactions to be studied in a more relevant environment. An intrinsic problem with developing new biological therapies is the difficulty in determining the affinity with which proteins will interact with intact cell surfaces. This methodology will be of interest to researchers developing new biological approaches for targeting cell surfaces in a wide range of diseases, including cancer.

1. Introduction

The development of pharmaceuticals is a costly enterprise with a significant attrition rate in the pre-clinical testing phase. New techniques are needed to provide information on the possible *in vivo* properties of novel biotherapeutics. A key step in the drug-development pipeline is evaluation of the affinity (dissociation constant, K_D) of the new biotherapeutic to its epitope. This is determined by measuring the on- and off-rate of the biotherapeutic to purified ligand immobilised (often *via* a spacer-molecule) to a planar surface. Unfortunately, however, this approach does not take into consideration the *in vivo* presentation of ligand. In the case of cell surface ligands these are found on the cell membrane in a milieu that includes diverse molecules in clusters, calthrin-coated pits, transmembrane proteins, lipids, GPI-anchored proteins and carbohydrates – all of which might potentially interfere with an interaction between a biotherapeutic and its ligand. We developed a system to assess the affinity of carbohydrate-binding proteins (lectins) to glycans directly at the cell surface and compared this with glycoproteins that had been immobilised on a planar surface.

The new biosensor system is based on the quartz crystal microbalance (QCM) technology and utilised adherent (tumour) cells grown on the surface of a gold sensor chip pre-coated with tissue culture compatible polystyrene. In the present study cancer cells were grown on the chip surface and were fixed in formaldehyde, the fixation process enabled regeneration of the cell surface between each interaction study and the cell chip to be used repeatedly, for approximately 20–30 experiments. Fixation in formaldehyde cross-links glycoproteins (Nirmalan et al., 2008), therefore, the formaldehyde fixation process might render some glycan epitopes less-accessible and reduce lectin binding but this is not considered to alter the overall binding pattern to cell surfaces (Brooks et al., 1998). Regeneration of non-fixed cells is a problem so we have worked on formalin-fixed cells as these cells represent a new model for the study of drug interactions at the cell surface. The system allows the study of interactions in the presence of other proteins that are normally found at the cell surface in contrast with conventional work using planar systems where, usually, simple, single protein/glycan entities are investigated. The applicability of the system is demonstrated with cancer cells and lectins although we consider the method to be of value for the

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evaluation of antibody–antigen and receptor–ligand interactions, and hope that this system is of interest to researchers working in diverse areas concerned with drug-development.

The QCM technique measures a change in resonance frequency (ΔF) as a mass accumulates on a quartz crystal (Sauerbrey, 1959), it is a label-free technique for studying biological interactions in real-time (Marx, 2003). Applications include off-rate screening, affinity measurement, epitope mapping and analysis of the thermodynamics of interactions, the technology has shown versatility and broad applicability for the analysis of molecular interactions (Pei et al., 2005; Cooper and Singleton, 2007; Pedroso et al., 2008; D'Ulivo et al., 2010; Johansson, 2010).

Previous studies have employed glycoconjugates immobilised onto sensor surfaces by amine coupling, physiosorption or by capture, for example, with streptavidin (Pei et al., 2005; Pedroso et al., 2008; Yakovleva et al., 2010). The analyte is typically injected over the sensor surface and changes in resonance frequency (ΔF) is monitored. The spatial arrangement of ligands at the sensor surface and concomitant binding has been the subject of a number of studies, for example by employing polymers in relation to enzyme binding properties (Marx et al., 2001).

Other recent studies have been concerned with measuring the kinetics of interaction between proteins and cell surfaces. These studies have often used a cell suspension and commonly trap the cells onto the sensor surface, by, for example, pre-coating the sensor with streptavidin and biotinylating the cells (Kuo et al., 2011), or by coating the surface of a biosensor with an antibody to facilitate the capture of cells on the surface (Saitakis et al., 2008). An issue with this approach, however, is that whilst being relatively fast and easy, it is best suited to cells that either grow as suspension cultures; or that are tolerant of being prepared as a cell suspension and is also dependent upon the method of cell capture. It is therefore of limited applicability for the analysis of cells that routinely grow as mono-layer culture and from a cell biology perspective it is desirable that methods be developed in which cells are grown as mono-layers prior to undertaking studies aimed at measuring cellular interactions. In an approach towards studying the entire cell surface, in a biosensor chip set-up, have shown that adherent cells may be grown and maintained on a gold sensor surface "treated chemically to render it hydrophilic" in a study concerned with microtubule arrangement and the effect of taxol and nocodazole treatment, however, the study did not address the interaction of biomolecules at the cell surface. Other reports have been concerned with the appropriate conditions for cell attachment and spreading (Modin et al., 2006; Fredriksson et al., 1998). The approaches that have used live cells in biosensor applications are not concerned with protein–cell interactions; rather they tend to focus on aspects of cell biology, for example cell adhesion and intracellular microtubule assembly. A recent review of this area shows the diverse approaches that have been taken to evaluate QCM sensors as tools for studying whole-cell interactions (Saitakis and Gizeli, 2012).

The applicability of the QCM-cell sensor system is shown here using a range of lectins including a recombinant form of the lectin: *Helix pomatia* agglutinin, HPA (Markiv et al., 2011) this is being developed in our laboratories as a tool for cancer prognostication and is also being pursued in a strategy aimed at biological targeting of cancer cells. Recombinant HPA was prepared either with a His-tag or a red fluorescent protein tag, and the binding properties to cancer cell membranes were compared with that of native HPA.

The method described here is suitable for investigations using a wide-range of biological systems and is an approach for measuring the kinetics of binding at the cell surface. We expect that the system will be of considerable interest to researchers targeting cell surface interactions with biological reagents as well as those developing the next generation of therapeutic antibodies.

2. Materials and methods

Chemicals were obtained from Sigma–Aldrich, Poole, Dorset, UK, unless otherwise stated. HPA lectin was purchased from Sigma–Aldrich and all other lectins were purchased from Vector laboratories, UK. All experiments were undertaken at 20 °C unless otherwise stated.

2.1. SW620 and SW480 cell culture

The human colorectal cancer cell lines SW620 (derived from mesenteric lymph node metastases) and SW480 (derived from Duke's B primary tumour of the colon of the same patient as the SW620 cells) were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Lonza, UK) supplemented with 10% (v/v) foetal calf serum (Biosera). SW620 cells have been shown to exhibit a metastatic phenotype when implanted subcutaneously into SCID mice whilst SW480 cells are non metastatic (Schumacher et al., 1996; Schumacher and Adam, 1997). Cells were kindly provided by Dr Loizidou (Royal Free and University College London). Cells were cultured using standard tissue culture techniques. Cells used for protein extraction were grown to 90–95% confluence, washed in PBS, mechanically detached in PBS using a sterile plastic cell scraper and centrifuged at 400 × *g* and were stored at −80 °C prior to protein extraction. Cell membrane proteins were prepared from the SW620 and SW40 as we aimed to compare the kinetics of lectin binding to the proteins with the kinetics of lectin binding to intact cells grown on the sensor surface. Cell pellets were re-suspended in lysis buffer (7 M urea, 4% (w/v) CHAPS, 1% (w/v) DTT, 2% (v/v) ampholytes and 2 M thiourea) and lysed by sonication using an ultrasonic probe (MS73 Status 200) at 40% power (Philip Harris Scientific, UK), with 5 pulses of 10 s each. Cellular debris was removed by centrifugation, using a Sorvall Super T21 centrifuge with a SL50T rotor, for 30 min at 11,000 × *g*, 4 °C and cell membrane proteins were prepared by ultracentrifugation (Lehner et al., 2003).

2.2. Preparation of cell-biosensor chips

Cells were grown on the chips and fixed in formaldehyde prior to evaluation in the biosensor. Cell culture compatible polystyrene coated QCM sensor chips were obtained from Attana AB (Stockholm, Sweden). The sensor chip was placed, face-up, in a well of a 24-well plate. An appropriate dilution of cells (to obtain 40,000 cells on the sensor surface) was added in 2 ml of media to the well and the plate was placed in an incubator at 37 °C (5% (v/v) CO_2 and 95% humidity) for 20 h. Following incubation, the cells were washed 3× with PBS and fixed for 30 min in fresh 3.7% (v/v) formaldehyde in PBS. To evaluate the cell coverage the nuclei were stained with To-Pro-3 and visualised using a confocal microscope (Saint-Guirons et al., 2007). For each cell type, two separate passages of cells were assessed. The QCM analysis was undertaken using a range of lectins, at different concentrations, each time in triplicate.

2.3. Preparation of protein-biosensor chips

Biosensor chips containing cell membrane proteins were prepared to compare with the cell chips (above). Polystyrene chips (Attana AB, Stockholm, Sweden) were fitted to the chip holder, pre-wetted by manual injection of 20 μl of 10 mM sodium acetate buffer, pH 5.0. 20 μl of cell membrane protein preparation (250 μg/ml) was injected into the chamber, wrapped in Parafilm and incubated for 3 h at room temperature and the chip was inserted into the QCM machine as described below. For each protein preparation, two separate passages of cells were assessed. The