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A novel label-free cell-based assay technology using biolayer interferometry

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ABSTRACT

Biolayer interferometry (BLI) is a well-established optical label-free technique to study biomolecular interactions. Here we describe for the first time a cell-based BLI (cBLI) application that allows label-free real-time monitoring of signal transduction in living cells. Human A431 epidermoid carcinoma cells were captured onto collagen-coated biosensors and serum-starved, followed by exposure to agonistic compounds targeting various receptors, while recording the cBLI signal. Stimulation of the epidermal growth factor receptor (EGFR) with EGF, the β_2-adrenoceptor with dopamine, or the hepatocyte growth factor receptor (HGFR/c-MET) with an agonistic antibody resulted in distinct cBLI signal patterns. We show that the mechanism underlying the observed changes in cBLI signal is mediated by rearrangement of the actin cytoskeleton, a process referred to as dynamic mass redistribution (DMR). A panel of ligand-binding blocking and non-blocking anti-EGFR antibodies was used to demonstrate that this novel BLI application can be efficiently used as a label-free cellular assay for compound screening and characterization.

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1. Introduction

Cellular signal transduction upon receptor stimulation is traditionally measured using classical biochemical assays, for instance by the quantification of second messenger molecules or determination of the phosphorylation status of a protein. However, a cellular response often involves activation of a complex network of signaling molecules. By focusing on a single readout other relevant signaling pathways may be overlooked. In addition, when screening orphan receptors there often is no knowledge of which signal transduction route is activated by the receptor. These challenges can be overcome by measuring an integral cellular response, instead of a single pathway. Advantages of such an integral approach are that no prior knowledge of the signal transduction cascade is required, and that responses often can be followed in time which allows evaluation of different signaling dynamics between compounds. There are commercially available label-free plate-based approaches that measure an integral outcome of cellular activation. Examples are the xCELLigence (Acea Biosciences) which measures a change in impedance upon stimulation of adherent cells over time, and the Epic (Corning) and BIND (SRU Biosystems) instruments that make use of the optical resonant waveguide grating technique.

Biolayer interferometry (BLI) is a label-free method that is commonly used to detect biomolecular interactions. In BLI the interference pattern of white light reflected from two surfaces on a disposable fiber optic-based biosensor is analyzed. The first surface on the biosensor tip consists of a layer of immobilized molecules, and the second surface is an internal reference layer. A change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that can be measured in real-time (Do et al., 2008). We developed a new BLI application that is compatible with living cells. Human A431 epidermoid carcinoma cells were captured on optical biosensors and exposed to a diverse set of agonistic compounds. This resulted in dose-dependent and compound-specific BLI signals. We show that the observed changes in BLI signal are the consequence of intracellular signaling affecting actin remodeling. Actin remodeling is thought to lead to changes in optical density by the redistribution of intracellular mass toward or away from the interface of the cell with the biosensor, a process referred to as dynamic mass redistribution (DMR) (Grundmann and Kostenis, 2015). Finally, we show that this unbiased label-free cell-based technique can easily be applied to functionally screen a panel of anti-EGFR antibodies for antagonistic properties.

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2. Materials and methods

2.1. Antibodies and compounds

The human antibodies specific to human EGFR zalutumumab and monoclonal antibodies (mAbs) 003, 005, 011 and 018 have been described previously (Bleeker et al., 2004; Dechant et al., 2008). IgG1-cMET specific for HGFR/c-MET was generated by immunizing HuMab mice (Medarex) with alternating NCI-H441 tumor cells and recombinant His-tagged extracellular domain of c-MET coupled to keyhole limpet hemocyanin (cMetECDHis-KLH). Antibody IgG1-cMET and mAbs 003, 005, 011 and 018 carried an engineered K409R mutation in the CH3 region (Labrijn et al., 2013) which does not affect binding or functional IgG1 properties. IgG1-cMET had an additional N297Q mutation (Tao and Morrison, 1989) which does not affect the antigen binding properties of IgG1-cMET. Nimotuzumab (Mateo et al., 1997), ICR62 (Modjtahedi et al., 1993), necitumumab (Lu et al., 2004), mAb 806, and IgG1-cMET were expressed in Freestyle™ HEK293-F cells (Invitrogen). Matuzumab (Murthy et al., 1987) was expressed in Expi293F™ cells (Gibco). IgG1-b12 directed against the envelope glycoprotein gp120 of human immunodeficiency virus type 1 (Roben et al., 1994) was expressed in CHOK1SV cells (Lonza). Antibodies were produced as human IgG1 with a κ light chain, purified using protein A affinity chromatography (MabSelectSuRe, GE Healthcare) and formulated in phosphate-buffered saline (B. Braun). Zalutumumab was produced by Lonza. Cetuximab (Erbitux) (Berger et al., 2011), panitumumab (Vectibix) (Yang et al., 1999) and trastuzumab (Herceptin) (Carter et al., 1992) were from Merck, Amgen and Roche respectively. Mouse anti-human EGFR IgG2a 528 (Kawamoto et al., 1983) was purchased from Calbiochem. Generation of the bispecific DuoBody molecule BsAb-cMETxb12 from the parental antibodies IgG1-cMET-F405L and IgG1-b12-K409R was performed as previously described (Labrijn et al., 2014), using an 1.3-fold excess of IgG1-b12-K409R in order to minimize the amount of residual agonistic IgG1-cMET-F405L homodimer in the DuoBody batch. The residual amount of IgG1-cMET-F405L homodimer was 0.3% as determined by cation exchange chromatography. Epidermal Growth Factor (EGF) and dopamine hydrochloride were obtained from Sigma-Aldrich.

2.2. Cell culture and capture of cells to biosensors

Amine-reactive second-generation biosensors (ForteBio) were incubated for 2 h at room temperature with a solution (0.1–1.0 mg/ml) of Collagen I, high concentration from rat tail (Corning) in water (B. Braun) followed by air-drying for at least 2 h. Human A431 cells (Deutsche Sammlung von Mikroorganismen und Zellkulturen) were cultured in RPMI 1640 (Lonza) containing 10% heat-inactivated donor bovine serum with iron, New Zealand origin (Gibco), penicillin (50 U/ml) and streptomycin (50 U/ml) (Lonza). Cells were harvested using Trypsin-EDTA (Lonza), washed with culture medium, and resuspended in undiluted 2 × Happy Cell RPMI Advanced Suspension Medium (Biocroi) to 10×10^6 cells/ml. 40 μl/well cell suspension was transferred to a 384 tilted-well plate (ForteBio). The collagen-coated biosensors were pre-wet for 10 min using 100 μl RPMI 1640 supplemented with penicillin and streptomycin in a 96 well half-area plate (Greiner Bio-One) inside an Octet HTX instrument (ForteBio), while the 384 tilted well plate was incubated at 30 °C at a shaker speed of 300 rpm. Capture of the cells to collagen-coated biosensors was then performed for 2000 s at 30 °C and a shaker speed of 300 rpm.

After capture of the cells the biosensors were transferred to serum-starvation medium (RPMI 1640 containing 0.1% serum, penicillin and streptomycin) and incubated overnight for at least 20 h in a cell culture incubator.

2.3. Cell-based biolayer interferometry

After overnight serum-starvation the collagen-coated biosensors containing cells were placed into the Octet HTX. Sample solutions containing compounds with or without inhibitors were prepared in serum-starvation medium in 384 tilted-well plates. After 10 min incubation of the sample plate at 30 °C with 300 rpm shaking, biosensors were dipped into serum-starvation medium with or without inhibitors for 1000 s in order to equilibrate. Next, the biosensors were dipped into sample solutions for 2000 s at 30 °C with 300 rpm shaking.

When indicated, the biosensors containing A431 cells were fixed with 3.7% formaldehyde (Sigma-Aldrich) in PBS for 15 min at room temperature. When indicated, the collagen-coated biosensors containing A431 cells were pretreated for 2 h in a cell culture incubator with serum-starvation medium containing the EGFR inhibitor tyrphostin AG-1478 (1 μM, Tocris), the β_2-adrenoceptor antagonist propranolol (1 μM, Tocris), the actin inhibitor cytochalasin B (10 μM, Sigma-Aldrich) or antibodies (15 μg/ml, 100 nM). Whenever inhibitors from DMSO stocks were used, the total amount of DMSO (Sigma-Aldrich) in the serum-starvation medium was kept constant at 0.05%, including solutions used for pretreatment, baseline and controls. The data traces shown in the graphs were obtained after subtraction of reference sensors exposed to serum-starvation medium with or without the appropriate inhibitors, using Data Analysis software v8.1 (ForteBio). The processed data were plotted using Prism 6 (Graphpad Software).

2.4. Cell viability

After cell capture using collagen-coated or untreated control biosensors, biosensors were incubated overnight in a cell culture incubator in serum-starvation medium containing 10% AlamarBlue (Invitrogen). The next day, fluorescence (excitation 515 nm, emission 615 nm) was determined using an EnVision Multilabel Reader (PerkinElmer). The two groups were compared with the Welch-corrected *t*-test using Prism 6.

2.5. Biosensor images

Images of the tip of the biosensor were taken while the biosensor was kept vertically above a 96 well half-area plate with the biosensor tip submerged in medium, using a Zeiss Axiovert 40 C inverted microscope in combination with a Canon G10 PowerShot digital camera.

3. Results

3.1. Capture of living cells to biosensors

In a typical biolayer interferometry (BLI) experiment using the Octet HTX platform, biosensors coated with a biocompatible matrix are used to interrogate 96- or 384-well plates by dipping the biosensors in the sample solution. This design implies that when adherent cells are to be captured from a cell suspension to the biosensor surface, the cells need to adhere to the bottom of the biosensor. Therefore the biosensors need to be coated with an agent that allows fast and firm attachment of living cells. To this end, the biosensors were coated with the extracellular matrix protein collagen type I.

The adherent human epidermoid carcinoma cell line A431 was suspended in 2 × Happy Cell Advanced Suspension Medium. The composition of Happy Cell Advanced Suspension Medium is designed to keep cells floating in suspension and was originally developed for 3D culture of cells. This property was exploited to

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