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Detection of apoptosis in cancer cell lines using Surface Plasmon Resonance imaging

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ABSTRACT

Induction of apoptosis in cancer cells by therapeutic agents is an important event to detect the potential effectiveness of therapies. Here we explore the potential of Surface Plasmon Resonance imaging (SPRi) to assess apoptosis in cancer cells exposed to therapeutic agents by measuring the cytochrome C release of apoptotic cells. Spots on the SPR sensor were coated with anti-cytochrome C, anti-EpCAM, anti-CD49e monoclonal antibodies and combinations thereof. Cells from the breast cancer cell line MCF7 were introduced into a flow cell, captured on a sensor surface and exposed to culture medium with and without paclitaxel. The cells were followed for 72 h. Clear SPRi responses were observed on the anti-EpCAM coated spots, indicating binding of the MCF7 cells with strong time and drug presence dependent increases in SPRi responses on the spots coated with both anti-EpCAM as well as anti-cytochrome C. This suggests a release of cytochrome C by the MCF7 cells in these specific locations. In addition offline experiments were performed where cultured MCF7 cells were exposed to complete culture medium with paclitaxel, Trastuzumab antibody and Trastuzumab T-DM1 (an antibody drug conjugate). The supernatant of these cells was analyzed and also their drug concentration dependent cytochrome C presence was detected. These preliminary results suggest SPRi to be a unique tool to measure real time response of cancer cells exposed to drugs or drug combinations.

1. Introduction

Surface Plasmon Resonance imaging (SPRi) can be used to measure the expression of antigens on the cell surface and antigens or proteins secreted by cells [12,13]. In recent years a larger number of potential therapeutic agents were identified of which only few entered into the clinic and even fewer showed therapeutic efficacy with a tolerable toxicity profile. All drugs have in common that they ultimately have to kill the cancer cells. These cells will undergo a process baptized apoptosis and during this process specific alterations in the cells take place. Measurement of apoptosis in cancer cells can therefore be used to screen the effectiveness of potential therapeutic agents on cancer cell lines with properties that can be related to the cancers to be treated [4] or to measure the response to therapy in patients [16]. A number of different compounds are excreted during apoptosis and one such compound is cytochrome C. Cytochrome C is a so called hemoprotein that is associated with the inner membrane of the mitochondrion where it is part of the electron transport chain [11]. Under normal conditions cytochrome C is bound to cardiolipin in the inner mitochondrial membrane. This prevents the initiation of apoptosis. Cytochrome C can become detached from cardiolipin in early apoptosis by its oxidization due to the production of mitochondrial reactive oxygen species. Cytochrome C plays an intermediary role in early apoptosis and upon release into the cytoplasm it binds apoptotic protease activating factor-1 (Apaf-1) and activates caspase 9 [6], which in term starts a cascade of events with apoptosis as the end result. As cytochrome C is highly water soluble it can easily dissolve into the cytoplasm and disperse throughout the cell. As apoptosis progresses cytochrome C will inevitably also leak from the cell. This makes the detection by SPR possible.

A variety of methods are described and are commercially available. Characteristics of apoptosis such as cell loss, nuclear morphology, DNA content, cell membrane permeability, mitochondrial membrane potential changes and cytochrome C localization and release are measured. However in order to detect all of these characteristics a number of components are mixed together in a complex and laborious protocol. Measurements of apoptosis are usually end point measurements and cells cannot be followed in real time. The most commonly used techniques to measure apoptosis are microscopy and flow cytometry [1–3,7,9,14,15,17,18]. Though robust and specific, these methods have their own unique challenges and downsides.

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SPR can be a potential alternative technique to study apoptosis. Previously it was shown that SPR can be used to detect apoptosis-associated genes [5], but this was not done using a cell sample. In a different study cells were brought into apoptosis and their morphological changes were monitored using SPR [8].

Here we explored if SPR can be used to detect early apoptosis by measurement of the apoptosis marker cytochrome C excreted in cell supernatants either by collecting the supernatant after exposure to different drugs added at different concentrations or by measuring the response of viable cells that were captured and exposed to drugs in real time in the SPRi instrument. In our experiments we used a cytostatic compound (Paclitaxel) a therapeutic antibody (Trastuzumab) and an antibody drug conjugate (ADC) (Trastuzumab T-DM1), available commercially as Herceptin® and Kadcyla® respectively from Genentech inc., South San Francisco, California, USA. Detecting early apoptosis using SPR would enable rapid therapy screening using live cells which would further contribute towards the development of personalized cancer therapy.

2. Materials and methods

2.1. SPRi

For SPRi analysis an IBIS MX96 SPR imager was used (IBIS Technologies BV, Enschede, The Netherlands). The height of the flow cell was 300 μm enabling a homogeneous injection of cells and cell medium. The obtained homogenous cell sample injection and larger volume of culture medium improve the cell viability over time.

2.2. CFM spotter

For ligand immobilization on SPR sensor surfaces the Continuous Flow Microfluidic (CFM) spotter was used (Wasatch Microfluidics LLC, Salt Lake City, Utah, USA) [10]. Ligand immobilization buffer was used to prime the CFM system and to dilute the desired ligands. The immobilization protocol lasted 30 min. The CFM spotter with a 6×8 print head configuration is capable of spotting up to 48 different ligands onto the sensor in a single run simultaneously under back and forth confined flow. The confined back and forth flow increases the efficiency of the spotting and avoids the risk of evaporation for contact and non-contact droplet based spotting methods.

2.3. SPR sensors

Easy2Spot® pre-activated G-type Senseye® sensors (Ssens BV, Enschede, The Netherlands) were used as SPR sensor surfaces. The sensors are delivered with a 100 nm hydrogel-like layer. This enables higher capacity coupling of ligands in the evanescent field and gives the ligands a level of mobility. The sensors are pre-activated for easy immobilization without using an additional EDC–NHS activation protocol.

2.4. Antibodies

Anti-cytochrome C (Biolegend inc., San Diego, California, United States of America) was used to capture cytochrome C, which is excreted by the cells when they are undergoing apoptosis. Anti-Epithelial Cell Adhesion Molecule antibody (anti-EpCAM, VU1D9) and Human epidermal growth factor receptor 2 antibody (anti-Her2) (both kindly provided by Immunicon, Huntingdon Valley, Philadelphia, USA) were used to capture cells based on their cell surface molecule expression. Anti-CD49e (BioLegend, San Diego, California, USA) was used as a negative control as MCF7 cells do not express this marker on their cell surface. Antibody Immunoglobulin G fragment crystallizable region (Anti-mouse IgG Fc) (BioLegend, San Diego, California, USA) was used to capture all antibodies present in the sample.

2.5. Apoptosis inducing agents

To induce apoptosis, paclitaxel (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), the therapeutic antibody Trastuzumab and the ADC Trastuzumab T-DM1 (kindly provided by Dennis Waalboer at the VU Amsterdam, The Netherlands) were used.

2.6. Ligand immobilization buffer

A 10 mM solution of sodium acetate (NaAc) immobilization buffer with pH 4.5 was made using anhydrous sodium acetate (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and acetic acid (Merck Schuchardt OHG, Hohenbrunn, Germany). First a 0.2 M stock solution was made of both components. Then from these stock solutions 1.93 parts of sodium acetate and 3.07 parts of acetic acid were mixed and finally 95 parts of ultrapure demineralized water were added. The pH was checked and if needed adjusted to pH 4.5.

2.7. System buffer

As system buffer the complete cell culture medium of the MCF7 cell line which was being analyzed was used, unless otherwise noted in the experiment description.

2.8. Cells

The cell line that was used for the experiments was the breast cancer cell line MCF7. MCF7 is an adherent cell line. To harvest MCF7 cells a trypsin protocol was used and the cells were resuspended in complete culture medium (RPMI 1640 + 10% fetal calf serum + 1% penicillin streptomycin + 1% L-Glutamine). RPMI 1640 was purchased from Lonza Group Ltd., Basel, Switzerland. Fetal calf serum and penicillin streptomycin were purchased from Sigma-Aldrich Chemie GmbH, Steinheim, Germany.

2.9. Deactivation agent

A 1% Bovine Serum Albumin solution (BSA) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in sodium acetate immobilization buffer was used as a deactivation agent. A stock solution of 2-aminoethanol (MP Biomedicals LLC, Illkrich, France) was used to create a 100 mM 2-aminoethanol solution with a pH of 8 and used as an extra sensor deactivation step after the initial BSA deactivation.

2.10. Sensor regeneration agent

As sensor regeneration agent a 200 mM solution of H_3PO_4 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was used. Sensor regeneration was performed for 1 min.

2.11. Detection of cytochrome C in supernatant samples of cultures treated with paclitaxel

To investigate if SPR is capable of detecting cytochrome C in culture supernatant, several flasks of MCF7 were cultured with various amounts of apoptosis inducing paclitaxel. One flask was without any addition of paclitaxel, the second had 1.5 μl (100 nM) of paclitaxel, the third had 3.0 μl (200 nM) and the last one had 6.0 μl (400 nM) added to 15 ml of complete culture medium respectively. The flasks with added paclitaxel were first cultured to 80–100% confluence before the compound was added by a medium refresh. After the addition of the drugs, the flasks were cultured for another 48 h at 37 °C and 5% CO_2. After these 2 days supernatants were removed from the flasks. An SPR sensor was prepared with varying spots containing: anti-cytochrome C (using a spotting solution of 20 μg/ml antibody in ligand immobilization buffer), anti-EpCAM as a negative control (using a spotting solution of 10 μg/ml