Kaufmann, K., Simmons, L., Herrmann, J., Schwär, G., Luniak, N., Müller, R.

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(2013) Biotechnology Letters, 35 (1), pp. 11-20.

Section: Bioprocessing and Bioengineering

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Authors: Katrin Kaufmann, Luke Simmons, Jennifer Herrmann, Gertrud Schwär, Nora Luniak and Rolf Müller

Katrin Kaufmann, Luke Simmons, Jennifer Herrmann, Gertrud Schwär, Nora Luniak:

Department of Pharmaceutical Biotechnology, Saarland University, Saarbrücken 66123, Germany

Katrin Kaufmann: Email kaufm@uni-trier.de

Luke Simmons: Email tl.simmons@mx.uni-saarland.de

Jennifer Herrmann: Email je.herrmann@mx.uni-saarland.de

Gertrud Schwär: Email g.schwae@mx.uni-saarland.de

Nora Luniak: Email n.luniak@mx.uni-saarland.de

Rolf Müller (corresponding author):
Department of Pharmaceutical Biotechnology, Saarland University, Saarbrücken 66123, Germany and
Department of Microbial Natural Products, Helmholtz Centre for Infectious Disease, Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) , Saarland University, Saarbrücken 66123, Germany
Email: rom@mx.uni-saarland.de

Tel.: ++49/681/302-70240
Fax: ++49/ 681/ 302-70202

Abstract

Using an in vitro cell-based assay in a flow-design, we have applied activity-guided screening to search for new bioactive compounds isolated from microorganisms. A first assay employs the stable expression of Nuclear Factor kappa B (NF-κB) while a second assay utilizes the glucocorticoid receptor (GR) coupled to green fluorescent protein. A specialized assay was implemented for both the translocation of NF-κB and to inhibit the translocation of cytokine-mediated NF-κB. In addition, we developed in a wide palette of cell lines used for a highly specialized GR-translocation assay to detect anti-inflammatory effects. This approach demonstrates the straight-forward combination of cell-based assays arranged with an automated fluorescence microscope. This allows for the direct sorting of extracts which are acting in a pharmaceutically interesting way. Initial results using this technique have led to the detection of new anti-inflammatory steroids from bacterial crude extracts.

Keywords

Activity-guided screening, anti-inflammatory steroids, glucocorticoid receptor, green fluorescent protein, Nuclear Factor kappa B

Introduction

New antibiotics and anticancer drugs are in high demand because of development of resistance in microorganisms and tumor cells (Demain and Sanchez 2009). This is causing a critical need to identify novel sources of bioactive molecules. The production and characterization of many new compounds from diverse prokaryotic sources is in progress (Simmons *et al*. 2005; Wenzel and Müller 2009; Garcia *et al*. 2009). As a result, high-content-screening has proven to be a versatile tool in drug discovery for the last ten years (Bickle 2010). In this paper, we describe how to investigate organic extracts exhibiting specific bioactivities and how to purify the fractions which are acting in the cell-based assays. This approach facilitates the detection of new active compounds, since only the active fractions are investigated. A wide variety of assay systems are used enabling this work to cover a diverse palette of pharmaceutically interesting effects. A significant part of the in vitro cell-based activity-based screening reported here aims to find new biologically active compounds from marine sponge-derived microorganisms.

Mammalian cell-based assays are usually adopted to target crude extracts from microorganisms displaying cytotoxic and antioxidative (reactive oxygen species, ROS) effects. Because these assays allow only coarse detection of biological activity, we applied two additional assays aimed to further elucidate a possible mode of action. One assay allows direct observation of Nuclear Factor κB (NF-κB) translocation or translocation inhibition. The other assay discovers glucocorticoid receptor (GR) signal transduction events.

For preliminary detection of cytotoxic effects of crude extracts, MTT assays were performed with mammalian cell lines. From the extracts which did not exhibit a cytotoxic behaviour, we tested for antioxidative effects using a modified DCFHDA (2', 7'-dichlorodihydrofluorescein diacetate, acetyl ester) assay (Degli 2002). The generation of ROS has many effects on the cell metabolism, for example, apoptotic processes or inflammatory

responses. ROS generation induced by certain substances can be measured by DCFHDA**.** When the DCFHDA is cleaved by the cytosolic esterases, the ROS oxidizes the DCF resulting in an increase in the fluorescence intensity. Antioxidants, namely N-acetylcysteine (NAC) or resveratrol (Wu *et al*. 2010, Holthoff *et al*. 2010), prevent the generation of ROS within the cell. NAC both interferes with ROS protecting cells against oxidative stress, and it refills the intracellular cysteine level to produce glutathione decreasing the presence of intracellular thiols (Staal *et al*. 1990).

In many cases, NAC also prevents NF-κB activation (Wu *et al*., 2010). NF-κB is a transcription factor that is localized and inhibited by IκB (inhibitor of NF-κB) in the cytoplasm in non-stimulated cells. Upon stimulation either by an oxidative or an inflammatory event, NF-κB is released from IκB, where it translocates into the nucleus and binds to NF-κB binding sites within the DNA to act as a transcription factor. Target genes of NF-κB are normally those involved in stress, immune, and inflammatory response, as well as apoptosis (Karin and Ben-Neriah 2000; Erlandsson *et al*. 2002). Grivennikov and Karin (2010) discuss the role of inflammation in tumorigenesis where inflammation can lead to production of ROS in immune cells and in pre-malignant cells by immune cell released cytokine induction. Thus, ROS and cytokines strongly promote tumor growth. Here, NF-κB, which is activated by cytokines, more specifically TNF-α or IL-1β, plays a pivotal role in regulating inflammatory events in tumor progression, and therefore serves as a crucial signal transduction step (Karin 2009; Karin and Ben-Neriah 2011).

A signal transduction protein which is clearly involved in inflammatory regulating processes is the glucocorticoid receptor (GR). GR is a cytoplasmic receptor that translocates into the nucleus after being activated by glucocorticoid hormones. Once inside the nucleus, GR activates or represses transcription, *in trans*, either by binding to specific DNA-sequences or by interacting directly with transcription factors. This effect generally leads to the suppression of immune and inflammatory responses (Heitzer *et a*l. 2007; Franchimont *et a*l. 2002). Steroid hormones act on the transcription of cytokines, TNF-α, and on direct or indirect activation of inflammatory-associated transcription factors, in particular NF-κB and activated protein-1 AP-1 (Kim *et al* 2004). A clear cross-talk between NF-κB- and AP-1 induction of cytokines, TNF-α and the GR, on protein- and on transcription level has been described (Van Boegert *et al* 2010).

In the first steps, the assay flow we describe here consists of the well-described MTT-assay and the DCFHDA-assay (Degli 2002). Many commercially available assays detect NF-κB -activation using luciferase as the reporter, yet there is a trend to develop assays for the observation of NF-κB translocation into the nucleus**.** In these not only the activation of promoter activity but also the translocation of NF-κB following a cytokine stimulus event can be observed by immunostaining in relatively simple end-point assays (Bertelsen 2006). In this paper, a commercially available cell line expressing green-fluorescent protein (GFP)-coupled NF-κB is implemented in a NF-κB inhibition assay to allow online detection in living cells using a high-content bioimaging system.

The first step in signal transduction of GR is the translocation of the activated GR into the cell nucleus. Agler *e*t *a*l. (2007) have shown translocation of yellow-fluorescent protein (YFP)-GR in Cos-7 cells in a high content approach. Here, we present the cell-by-cell observation of GR-EGFP translocation into the nucleus after

stimulation with the artificial corticosteroid dexamethasone (Dex). We describe an assay based on stably transduced cell lines expressing the GR-EGFP fusion protein using an online detection of whole cells with a high-content bioimaging system.

In Simmons *et al*. (2011), activity-based discovery of new bioactive steroids was demonstrated with an emphasis on the compound isolation. Here, we focus on the exact cell-based assay approach to show the ability of this assay system to reveal new compounds in a fast and targeted way using a high-content approach, as all assays can be performed subsequently or in parallel with a set of whole cells and automated read-out supporting the compound isolation.

Materials and Methods

Cell lines

For the MTT assays, L929, a mouse subcutaneous connective tissue fibroblast cell line was used growing in Roswell Park Memorial Institute (RPMI) 1640 medium, 10% (v/v) fetal calf serum (FCS).

The CHO/ GFP-NF-kBp65 cell line obtained from Affymetrix (Santa Clara, CA) was used for monitoring the activity of NF-kB transcription factor in cell-based assays.

For the ROS-assay and for the stable expression of GR, U2OS, an osteosarcoma cell line was used, which is growing adherent and is easily to monitor because of its plane growth and its rather large size with distinct cell shape (Gasparri *et al*. 2006). The cells were growing in McCoy's 5a Medium, 10% (v/v) FCS.

In addition, the cell lines listed in **Table 1** were transduced for expression of GR-EGFP.

For transfection with the lentiviral vectors, 293T/17 cells (a highly transfectable derivative of the human primary embryonal kidney cell line 293 were cultured in DMEM high glucose (4.5 g/l) medium containing 10% (v/v) FCS.

Cell culture media and FCS was obtained from Gibco, Darmstadt.

Plasmids

The lentiviral vector pFUGW (Lois *et al*. 2002) was used as the expression vector for GR-EGFP. The *gr*-gene gained from cDNA from human placenta was inserted BamHI/BshTI with the oligonucleotides NR3C1-BamHI-for (AAAGGATCCATGGACTCCAAAGAATCATT) and NR3C1-BshTI-rev (AAAACCGGTTTTTGATGAA-ACAGAAGTTT) generating NR3C1into pFUGW obtaining pFUGW-NR3C1. pCMVΔR8.91 was cotransfected as a packaging vector which is coding for GAG, POL and TAT and ENV, with the LTRs eliminated, pCMVG was cotransfected as an expression vector for the VSVG envelope (Zufferey *et al*. 1997) and (Naldini *et al*. 1996).

MTT Assay

The MTT assay was performed as described in Simmons *et a*l. (2011)

ROS Assay (DCFHDH)

U2OS cells were seeded at 10^4 cells per well into an imaging plate. The next day cells were washed with KRH buffer (119 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM KH_2PO_4, 10 mM HEPES and 2 mM D-glucose, pH 7.4) and incubated with 100 µl 10 µM 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (DCFHDA, Sigma-Aldrich) in KRH-buffer for 1 h at 37°C. Next, they were washed twice with KRH buffer and incubated with the crude extracts or test fractions in a volume of 75 µl for 1 h at 37°C. Induction of ROS production took place with 25 µl 200 µM *tert*-butyl-hydroperoxide (tBHP, Sigma-Aldrich) for 20 min at 37°C. 4 µg Hoechst 33342/ml (Molecular Probes, Darmstadt) was added for nucleus staining. A live cell DCFHDA-measurement was carried out with the Pathway Bioimager 855 (BD, Heidelberg).

Stable transduction of cell lines

For the transfection of mammalian cell lines with an expression vector encoding a fusion protein of the glucocorticoid receptor and EGFP, we employed the lentiviral transduction method by (Lois *et al*., 2002) based on the calcium phosphate transfection technique (Jordan and Wurm 2004) and designed according to Roessler and Thiel (2009). 293T/17 cells were seeded at 10^6 cells in a T25 cell culture flask, in DMEM 10% (v/v) FCS and incubated for 24 h at 37°C, 5% (v/v) CO_2. The cells were then transfected by calcium phosphate method. For every transfection, two 12 ml polystyrene vials were prepared. In the first vial, 550 µl 2 x HBSS (0.137 M NaCl, 5.4 mM KCl, 0.25 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1 mM $MgSO_4$, 4.2 mM $NaHCO_3$, pH 7.05-7.12) was prepared. In the second vial, 10 µg DNA was carefully mixed with 55 µl 25 mM $CaCl_2$ before sterile H_2O was added to make 500 µl. Medium aspirated from the cells was replaced by 4 ml fresh medium, containing 2.5µl 10 mM chloroquine/ml (Sigma-Aldrich). The $CaCl_2$/DNA solution was slowly pipetted into the 2 x HBSS with a long Pasteur pipette while bubbling the 2 x HBSS with a second long Pasteur pipette. The bubbling was maintained for a few seconds after adding the $CaCl_2$/DNA-solution. 500 µl Ca_2PO_4/DNA was dispersed over the cells. The flasks were incubated at 37°C, 5% (v/v) CO_2 for 8 h, then the cells were washed twice with PBS and incubated with 4 ml DMEM, 10% (v/v) FCS for three days. U2OS and all other infected cells were seeded at 5 x 10^3 cells per well in a 96 well imaging plate or at 10^6 cells in a T25 cell culture flask in Mc Coy´s 5a Medium, 10% (v/v) FCS and grown for 24 h at 37°C, 5% (v/v) CO_2. After three days, the T293 cells secreted virus into the supernatant. The supernatant was filtered through a 0.45 µm filter and filled up to the required volume for infection. No more than one third of the virus stock was used for this. Then, 8 µg polybrene/ml (Sigma-Aldrich, stock solution 8 mg/ml, filtered via a 0.25 µM filter) was added and, after medium aspiration, 100 µl per well or 4 ml per flask virus stock was added to the cells to be infected. The medium was changed without washing the cells after 8 h. Three days later, cells were seeded 10^3 cells per well into a 96 well imaging plate (BD, Heidelberg). Every two to three days cell nuclei of one row were stained by Hoechst 33342. Fluorescence of transduced cells was observed and compared to the whole cell number defined by Hoechst. Transduction efficiency was shown to be at nearly 100 %. Upon sequential freezing, thawing and cell cultivation over several weeks retained the constitutive expression of the GR-EGFP-fusion protein and the fluorescence intensity. The stable cells are grown in McCoy's 5a medium, 10% (v/v) FCS.

Treatment of transduced cells expressing GR-EGFP

Cell lines stably expressing GR-EGFP were seeded at 5 x 10^3 cells per well in a 96 well imaging plate. The next day, treatment with either 100 nM Dex (Sigma-Aldrich) or different concentrations of the indicated substances took place for 1 h. Then cells were stained with Hoechst and GFP-intensity was measured by Pathway Bioimager. Hoechst staining was carried out by first washing cells with PBS (phosphate buffered saline, 100 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4), then adding 4% (v/v) paraformaldehyde in PBS for 10 min. This was followed by washing with PBS, incubating with Hoechst, 1 µg/ml, for 15 min in the dark, aspirating staining solution and adding PBS. The cell nuclei were stained with Hoechst to allow a segmentation of cytoplasm and nucleus and GFP-fluorescence in cytoplasm and nucleus.

Treatment of CHO-NF-κBp65-GFP cells

The CHO/ GFP-NF-kBp65 cell line was treated following the prescription in Simmons *et a*l. (2011).

Image and data analysis

Images for screening were obtained by the automated fluorescence microscope Pathway 855 from BD. All pictures were gathered in 200 fold amplification using a microscope objective of 20 x U-Apo340 (Olympus, NA 0.75). Per well a field of 2 x 2 (500 to 1000 cells or regions of interest (ROIs)) was analyzed. The pictures were gathered by a laser-based autofocus. The Hoechst-stained cell nuclei were determined with a 360/10 excitation filter and a 435 LP emission filter. In the DCFHDA-assay, each well was measured with a 488/10 nm excitation filter and a 515 nm emission filter. Each experiment was accomplished at least in two separate wells to obtain two average values. For the determination and prevention of DCFHDA oxidation, all values were normalized and controls set to 0 and 1, respectively. The standard deviation was calculated and is shown in the bar graphs. The translocation assays cytoplasm and nucleus fluorescence were determined separately with a 488/10 excitation filter and a 515 LP emission filter. Each experiment was accomplished in at least two separate wells to obtain two average values. The ratio between nucleus fluorescence and cytoplasm fluorescence was calculated (nuc/cyto). Then values between 0 and 1 were calculated ((x-min)/max-min)). The average between the well data and the standard deviation was estimated and shown in the bar graphs.

Results and discussion

MTT and ROS Assays (DCFHDA)

Following the work of Simmons *et a*l. (2011), and Iizuka et al. (1998) , we described the isolation of a marine bacterium, *Actinomadura* sp. SBMS009, from the sponge *Suberites japonicas* and the extraction procedure for purification of some of its metabolites. The ethyl acetate extracts were washed and the resultant organic fraction was then fractionated on a silica gel flash column and eluted sequentially with hexane/ethyl acetate (2:1 v/v; 1:1 v/v; 1:2 v/v), 100 % ethyl acetate, ethyl acetate/methanol, (2:1 v/v; 1:1 v/v; 1:2 v/v) and 100% (v/v) methanol. These fractions are called L0, L3, L7, L9, L10, L11 and L12, respectively. (The final fraction - 100% methanol - is not included in these numbered fractions.) The first steps in the purification process were guided by the MTT and ROS assays. The extracts L0, L3, L7, L9, L10, L11 and L12 were tested for their cytotoxic properties. No cytotoxic effects could be found in 2.5 mg/ml and 0.25 mg/ml crude extracts using L929 cells as reported in (Simmons *et al*. 2011). As cytotoxicity assays do not reveal all biologically active compounds, we decided to apply an antioxidative assay for detection of potential drugs with cell recovering effects. The anti-oxidative

assay we describe here is based on the oxidation of DCFHDA, which is cleaved by cellular esterases within the cell by reactive oxygen species (ROS). U2OS cells were loaded with DCFHDA. Then extracts of the *Actinomadura* sp. SBMS009, or resveratrol (BioTrend, Cologne) as a control, were added and the cells were incubated with tBHP to induce ROS production. From Figure 1, it can be seen that the cells pre-incubated with resveratrol have much lower ROS production than the control cells. From the extracts tested, only L0, L3, L7 and L9 showed a recovery effect on ROS production by inhibiting the tBHP-induced oxidation of DCFHDA.

NF-κB anti-inflammatory assay

Antioxidants often prevent inflammatory processes in the cell (Grivennikov and Karin 2010). One global player in inflammatory processes is NF-κB. In the assay described here, we show the prevention of one of the crucial steps in the activation procedure of NF-κB, its translocation into the nucleus. The cell line CHO-GFP-NF-κBp65 was stimulated with interleukine-β (IL-1β, PromoKine, Heidelberg) at 20 ng/ml to induce translocation of NF-κB into the nucleus. We tested whether the IL-1β induced translocation could be retained. As shown in Figure 2, NAC and Dex prevented IL-1β from being induced by NF-κB-translocation into the nucleus. NAC as an antioxidant thiol blocks the cytokine-mediated activation of NF-κB (Staal *et al*. 1990). Dex was used before the following GR assay was applied. From the extracts tested, the only compound that could reproducibly inhibit the translocation of NF-κB in the ROS-assay more than the Dex was the fraction L9. The example in Figure 2 (photographs) show that IL-1β induced translocation of NF-κB is completely inhibited by either NAC as a canonical translocation inhibitor or by fraction L9. As we worked with bacterial material, we also looked for inflammatory action of the fractions, but no inflammatory effect was detected by incubation of the line CHO-GFP-κB cells with the fractions alone (data not shown).

From extract L9, which showed activity in the ROS assay and in the NF-κB assay, subfractions from RP18-HPLC/MS-analysis (Simmons *et a*l. 2011) were again subjected to the NF-κB-assay. Here, only fraction L9B showed an anti-inflammatory effect (data not shown). Further purification of fraction L9B using a RP18-HPLC/MS semi-preparative column (Simmons *et a*l. 2011) resulted in the characterization of diverse compounds. Using the prescription from Simmons *et al.* (2011), we tested the isolated compounds on NF-κB, showing an inhibitory effect on NF-κB translocation.

GR anti-inflammatory assay

As the isolated compounds isolated by the assay flow described above revealed a steroid character (Simmons *et al.* 2011), we added the GR-assay described below. Subsequent screening resulted in isolation of three new anti-inflammatory compounds, bendigole D, E, and F, as described (Simmons *et al.* 2011).

Many agents are described which directly interfere with NF-κB**.** Still**,** the GR is another interesting point of interaction with NF-κB, as was shown also here using Dex as inhibitor for NF-κB translocation. It is known that GR shows cross-talk with NF-κB on cytokine level (Adcock *et al* 2001). It inhibits for instance TNF-α production on protein level (Bogaert *et al*. 2010), e.g. interfering with the MAP kinase cascade, or on nuclear level, inhibiting the transcription of NF-κB-responsive genes (Yamamoto *et al*. 2001). Taking this into consideration, we studied the influence of the isolated compounds Bendigole D-F on the GR-pathway in addition to the NF-κB-pathway.

The cell lines U2OS, HaCaT, SH-SY5Y and stably expressing GR-EGFP were stimulated with Dex. The ratio of GFP-intensity in the nucleus and in the cytoplasm was calculated (Figure 3). The comparison of translocation effects is demonstrated by the photograph shown in Figure 3. Bendigole D, E and F showed a clear GR-translocation activity, although less pronounced and only at higher concentration compared to Dex as a canonical inducer of GR-translocation. Bendigole F had the most apparent GR-translocation effect in all tested cell lines; bendigole E and bendigole D also triggered the translocation of GR into the nucleus. For the GR-translocation assay, the effect of all three steroids were most evident in the SH-SY5Y cell line (shown in Figure 3), where translocation of GR-EGFP into the nucleus is shown. The table implemented in figure 3 shows a comparison of the translocation (measured as the fluorescence intensity ratio) in the cell lines U2OS, HaCaT and SH-SY5Y. We used the purified bendigoles D-F for the final experiments described in Simmons *et al*. (2011).

The concentration of the steroids that is necessary for induction of a clear effect is rather high in comparison to pharmaceutically applied agents. This may explain the difficulty to gain a dose-response-curve with the tested substances. Nonetheless, we could show the translocation effect with near all adopted concentrations. The translocation abundance is diverse by using different concentrations and different cell lines, but the event itself is shown in each experiment. This may be due to aggregation of molecules in a higher concentration but also because we use whole cells that behave slightly differently in independent experiments, depending e.g. on the passage number. Although we used living cells for the assay, we had to stop the translocation event at a certain point of time in all cells for Hoechst-staining. This means the signal transduction procedure is observed possibly only in a few cells. Further experiments showed, that after an incubation time longer than 30 min, GR translocates back to the cytoplasm. GR dynamically translocates between cytoplasm and nucleus, a shuttling back occurs especially with those GR disposed of ligand and not binding to DNA (Vandevyer *et al.* 2012). This dynamic process of GR-shuttling may contribute to an unequal distribution of GR within a cell population, so that only an average is being measured.

We demonstrate here that bendigole D, E and F induce the translocation of GR into the nucleus of mammalian cell lines, which indicates an anti-inflammatory response. The concentration at which bendigole D, E and F induce GR-translocation is at least 25-fold higher than the concentration of the canonical artificial GR-activator Dex. Of the three bendigoles tested, only bendigole F showed an inhibition activity towards the NF-κB-translocation in a 2500-fold concentration in comparison to Dex (Simmons *et al*. 2011). This is probably due to corticosteroids not interacting with NF-κB directly but through cross-talk that takes place either by protein-protein interaction of the GR with NF-κB itself or with a certain NF-κB-inhibitor, like an IκB (Adcock and Caramori 2001). This may be due to a direct effect of bendigole F on NF-κB but is not examined further within this assay system. GR as well as NF-κB is expressed ubiquitously in nearly all tissues but especially the GR is regulated in a genomic or non-genomic manner in different ways dependent on the tissue (Heitzer *et al*. 2007; Rogatsky *et al*. 2003). This explains the diverse effects in diverse cell lines. Figure 4 shows a summary of possible actions of GR and NF-κB within the cell upon stimulation by Bendigole as well as the influence of IL-1β and ROS.

Conclusions

The developed assay system described here is effective for detecting bioactive compounds extracted from microorganisms as shown by the directive isolation of bendigoles D-F. The first assays performed here are primary assays to sort fractions, followed by more specialized NF-κB-translocation inhibition and the GR-translocation assays. The raw assays, such as the MTT and ROS assays are easy to perform and evaluate. This means that the sorting of extracts towards active fractions can be easily achieved. It should be pointed out here that by this assay approach we also found non- or low-cytotoxic compounds, whereas often only those extracts or compounds acting in a cytotoxic way are studied further to find new antibiotics or anti-cancer drugs (Weissman and Müller 2009). The more specialized assays allow elucidate more precise effects of the newly detected sub-fractions and compounds.

As translocation assays are commonly performed using immunostaining (Bertelsen 2006), our assay has the advantage of cells stably expressing a fusion protein with (E)GFP. In the approach presented here, we used a commercially available cell line for the anti-inflammatory NF-κB-assay. In addition to the anti-inflammatory assay, an inflammatory NF-κB assay can easily be performed. This is useful for measuring (anti-) cancer agents or antibacterial agents, as NF-κB is activated by a variety of external and internal stimuli (Pahl 1999).

To achieve a significant step in precise elucidation of signal transduction pathways, we developed the GR-assay which can be used for the observation of specific anti-inflammatory processes. Here, we have the expression vector in hands and the GR-translocation assay can be performed with a wide palette of cell lines stably expressing GR-EGFP without any selection additive in the medium. This facilitates a broad spectrum of diverse cell lines and is important because the GR action varies depending on the tissue (Kim *et al.* 2004). It allows for the action of new compounds on different cancer forms to be examined. In addition to the described cell lines stably expressing GR-EGFP, we were able to assemble a set of different cell lines for expression of GR-EGFP, including: HaCaT; L929; NIH3T3; SH-SY5Y; U2OS; A431; HMEC; CHO-K1; cos-7; HSF-1; and A549 cells. This allows the performance of this functional assay to be performed in many different tissue classes. The GR-assay is particularly useful for high-throughput approaches because of the easy-to-handle cell lines and the short assay time. More specifically, this assay only needs seeding of the cells, incubation with the substances, and gathering pictures automatically.

This “sorting by purification approach” is available for isolating of compounds from diverse sources in an activity-guided way by diverse combination of the assays introduced here. The assays can be performed in a flow or even in parallel with a set of whole cells, automated read-out serves sorting of fractions in an efficient way.

Acknowledgements

We thank Gerald Thiel for precious advice in lentiviral methods and the lentiviral vectors pFUGW, pCMVΔR8.91, and pCMVG. Research in R.M.`s laboratory is funded by the Bundeministerium für Bildung und Forschung and the Deutsche Forschungsgemeinschaft.