Monitoring intracellular Ca\textsuperscript{2+} fluxes and cAMP with primary sensors from Lonza

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- Luminescence-based calcium measurements in HUVECs utilizing Clonetics™ primary sensors
- cAMP was detected in hMSC with the help of Poietics™ primary sensors
- BMG LABTECH luminescence reader used to monitor dose-dependent responses

Introduction

Primary cells allow for a higher predictability of drug reactions in humans. These cells endogenously express relevant drug targets at physiological level and genuinely carry the components required for specific signal transduction. They can be derived from the actual tissue of interest. These are significant advantages over immortalized cell lines, which may be derived from irrelevant tissue, be of nonhuman origin, and often express transfected drug targets at non physiological levels. For these reasons, there is a growing demand for primary cells in drug screening and hit validation.

In this application note we show that primary human mesenchymal stem cells can be used in high-throughput formats (i.e. 96-well and 384-well plate) to monitor changes of intracellular cAMP concentration. For the monitoring of Ca\textsuperscript{2+} fluxes human umbilical vein endothelial cells (HUVEC) proved to be useful.

Materials & Methods

- 96-well and 384-well white microplates from Corning
- pGloSensor\textsuperscript{TM}22F cAMP plasmid & GloSensor cAMP reagent from Promega
- Poietics™ human mesenchymal stem cells (hMSC) from Lonza
- Ionomycin, ATP, thrombin, histamine from Sigma
- Neurotensin was from Bachem
- Clonetics™ primary sensors - HUVEC calcium biosensor from Lonza

Production of hMSC cAMP biosensor

Poietics™ human mesenchymal stem cells (hMSC) were transiently transfected with an expression plasmid encoding the GloSensor\textsuperscript{™}-22F using the Amaxa\textsuperscript{™} 96-well Shuttle\textsuperscript{™} Nucleofector\textsuperscript{™}. The transfected hMSCs were incubated after Nucleofection\textsuperscript{™} in a humidified tissue culture incubator (37°C, 5 % CO\textsubscript{2}) for 6 hours. Right before freezing the cells were loaded with 10 μM native coelenterazine for 2 hours. Subsequently the cells were frozen in vials in cryoprotective agent.

Production of HUVEC calcium biosensor

Clonetics™ HUVEC were transiently transfected with an expression plasmid encoding i-Photina\textsuperscript{R} using the appropriate Amaxa\textsuperscript{™} 96-well Shuttle\textsuperscript{™} Nucleofector\textsuperscript{™} kit and the Amaxa\textsuperscript{™} 96-well Shuttle\textsuperscript{™} Nucleofector. The transfected HUVECs were incubated after Nucleofection\textsuperscript{™} in a humidified tissue culture incubator (37°C, 5 % CO\textsubscript{2}) for 6 hours. Right after freezing the cells were loaded with 10 μM native coelenterazine for 2 hours. Subsequently the cells were frozen in vials in cryoprotective agent.

Detection of intracellular calcium release or intracellular cAMP

In order to perform the Ca\textsuperscript{2+}-assay or the cAMP assay cryopreserved cells were thawed, seeded on a 96-well or 384-well plate, and were allowed to recover overnight. 4 hours after thawing medium was exchanged for HEPES buffered medium to recover the cryoprotective agent. The plate was then ready to be measured.

Assay Principle

- cAMP Biosensor assay
- Calcium Biosensor assay

Materials & Methods

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Production of HUVEC calcium biosensor

Clonetics™ HUVEC were transiently transfected with an expression plasmid encoding i-Photina® using the appropriate Amaxa™ 96-well Nucleofector™ kit and the Amaxa™ 96-well Shuttle™ Nucleofector. The transfected HUVECs were incubated after Nucleofection™ in a humidified tissue culture incubator (37°C, 5 % CO\textsubscript{2}) for 6 hours. Right after freezing the cells were loaded with 10 μM native coelenterazine for 2 hours. Subsequently the cells were frozen in vials in cryoprotective agent.

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Results & Discussion

Ca²⁺ Assay
The reaction of the flash luminescence was followed over time (Fig. 3). Injecting the stimulating agonist while recording of luminescence intensity is on-going. Calcium release was triggered by injection of histamine (50 μM final concentration).

Instrument settings

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<th>Calcium assay</th>
<th>Omega series</th>
<th>CLARIOstar®</th>
<th>PHERAstar® FS</th>
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The assay system is well suited to generate pharmacologically relevant data for compounds that trigger calcium dependent signalling. Agonists for different classes of GPCRs (histamine receptors, purinergic receptors, neurotensin receptors) clearly show dose-dependent responses with the HUVEC calcium biosensor yielding EC50 values consistent with published data (data not shown).

cAMP Assay
The functional expression of the GloSensor™ protein in hMSC reactivated from frozen state was demonstrated by treating the cells with forskolin. Result is a clear dose-dependent response with an EC50 consistent with published data (Fig. 4).

Conclusion
The ready-to-use cell-based assay systems from Lonza in conjunction with a microplate reader from BMG LABTECH are an excellent tool to evaluate drug effects on signaling in primary cells.