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Cell-based Assays Using Hard-to-transfect Cell Lines and Transiently Transfected Primary Cells

Corinna Thiel[1], Ann Siehoff[1], Kristin Atze[1], Sandra Carsten[1], Alina Morsch[1], Steffi Franke[1], Mathias Kühn[1], Brock Binkowski[2], Pete Stecha[2], Jey Cheng[2], Elke Lorbach[1], Sonja Spicker[1] and Nicole Faust[1]
[1]Lonza Cologne AG, Nattermannallee 1, 50829 Köln, Germany; [2]Promega Corporation, 2800 Woods Hollow Road, Madison, Wisconsin, USA

1. Abstract

Cell-based assays have gained widespread popularity in the preclinical drug discovery process. Standard cell lines such as HEK-293, CHO-K1 or HeLa are routinely used for cell-based high-throughput screens. Results acquired using these cell systems are frequently misleading. Reporter cell lines and primary cells with greater physiological relevance, such as HUVEC, AoSMC or stem cells provide powerful new tools to increase the predictive value of data gained from cell-based assays.
Reliable transfection of these cell types has been challenging due to the lack of appropriate transfection methods. The Amaxa® Nucleofector® Technology enables the generation of stable transfectants of various hard-to-transfect cell lines and highly efficient transient transfection of primary cells. Using this technology, we successfully introduced Promega reporters and biosensors into difficult-to-transfect cell lines and primary cells to monitor molecular signaling events. To allow for flexible and fast applications, cells carrying reporters and biosensors can be cryopreserved in large numbers in vials or ready-to-use on 96-well plates.
Our results demonstrate that the Amaxa® Nucleofector® Technology in combination with Promega reporter systems facilitates cell-based assays in hard-to-transfect cell lines and primary cells signifying valuable new possibilities for target validation and secondary or even primary HT-screenings.

2. Introduction

Primary cells are becoming a more and more accepted tool for cell-based assays. However, the label-free methods currently used for their analysis cannot properly address signal transduction at the molecular level. In an effort to make primary cells more widely usable for cell-based assays, we combined Lonza's expertise with primary cells and their transfection and Promega's expertise in cell signaling.
In the work presented here, we successfully used a cAMP biosensor (pGloSensor™) as well as CREB- and NFAT-inducible luciferase reporters in different primary cells, such as Human Umbilical Vein Endothelial Cells (HUVEC), Human Lung Microvascular Endothelial Cells (HMVEC-L), and Human Mesenchymal Stem Cells (hMSC). Primary cells transiently transfected with either the biosensor or one of the inducible luciferase reporter systems showed a significant response after treatment with forskolin and PMA/ionomycin or to physiological stimuli such as histamine.

3. Materials and Methods

Optimization of Nucleofection® Protocol for hMSCs on the 96-well Shuttle®:

Poietics® Human Mesenchymal Stem Cells (hMSC, Lonza PT-2501) isolated from bone marrow were cultured and expanded according to Lonza Protocols. Nucleofection® was performed with 50,000 cells and 0.4 µg pmaxGFP® Vector per sample using program 96-FF-104 and 96-EW-104 on the Amaxa® 96-well Shuttle® System. Transfection efficiency and CD44 marker expression were analyzed by flow cytometry. Viability was analyzed using the Vialight® Plus Kit (Lonza).

Analysis of intracellular cAMP levels in primary cells and cell lines using pGloSensor™ Plasmids:

K-562 (ATCC®), Human Umbilical Vein Endothelial Cells (HUVEC, Lonza CC-2517) and hMSC were transfected with the pGloSensor™-20F cAMP Plasmid (GloSensor 1), pGloSensor™-21F cAMP Plasmid (GloSensor 2.1) or pGloSensor™-22F cAMP Plasmid (GloSensor 2.2) (Promega) using the appropriate Amaxa® 96-well Nucleofector® Kit and the Amaxa® 96-well Shuttle® System. 17 – 20 hours after Nucleofection®, cells were equilibrated for two hours with GloSensor™ cAMP Reagent (Promega) according to the Promega protocol. Cells were stimulated with various forskolin concentrations and luminescence was analyzed for up to 30 minutes at various temperatures in a microplate reader.

Analysis of intracellular NFAT signaling in primary cells:

HMVEC-L and HUVEC were transfected by Nucleofection® with an NFAT-inducible luciferase construct (pGL4.30[luc2P/NFAT/Hygro], Promega) using optimized protocol conditions for the respective cell type. Six hours after Nucleofection®, HMVEC-L were stimulated with various PMA/ionomycin concentrations for a 16 hour time period. HUVEC were stimulated with different histamine concentrations two hours after Nucleofection® for a four hour time period. Induction of chemiluminescence was measured using the Promega BrightGlo™ Luciferase Assay System.

Analysis of intracellular cAMP signaling in primary cells:

HMVEC-L were transfected by Nucleofection® with a cAMP-inducible luciferase construct (pGL4.29[luc2P/CRE/Hygro], Promega), using optimized protocol conditions. Two hours after transfection, cells were stimulated with different forskolin concentrations for four hours. Cells were analyzed for forskolin response using the BrightGlo™ Luciferase Assay System (Promega) and for viability using the CellTiter-Blue® Cell Viability Assay (Promega).

4. Results

Optimization of Nucleofection® Protocol for hMSC

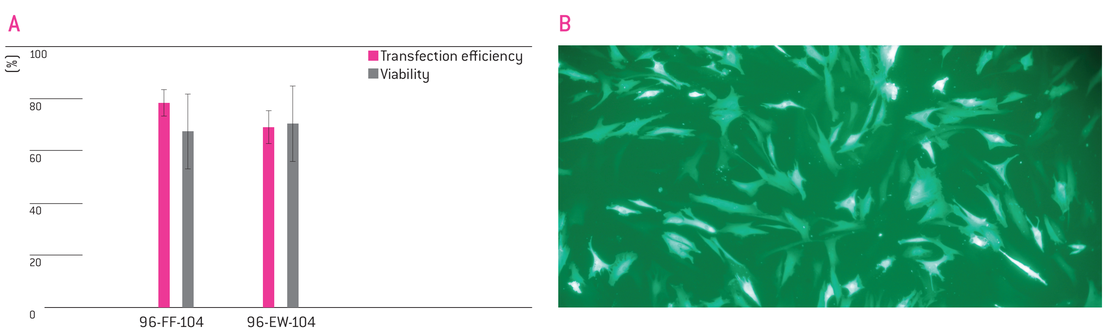

Figure 1. Poietics® hMSC were transiently transfected with pmaxGFP® Vector and Amaxa® 96-well Shuttle® System (24 hours). A) Transfection efficiency and viability. B) GFP Fluorescence.

Glosensor™ in primary cells

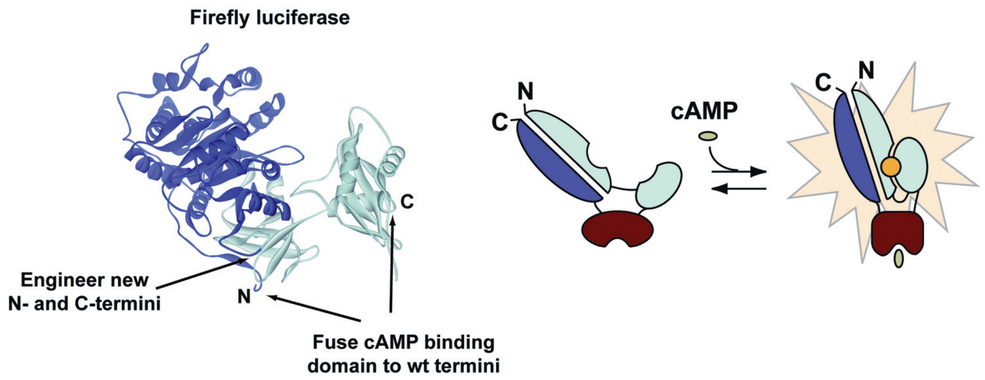

Figure 2. GloSensor™ Technology overview

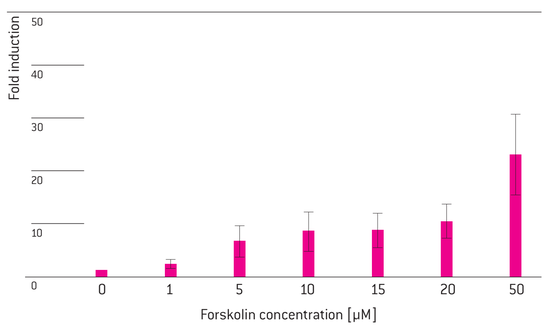

Figure 3. K-562 were transiently transfected with pGloSensor™-20F cAMP Plasmid and stimulated for 10 min with various forskolin concentrations (18 hours after Nucleofection®). Stimulation and data collection at 32°C.

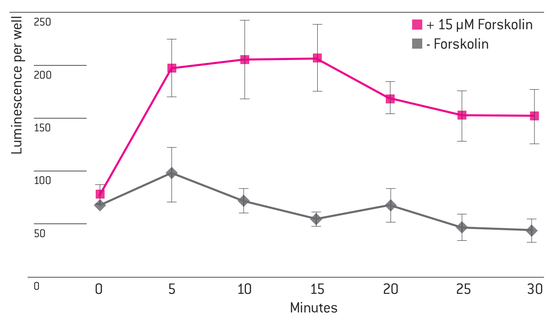

Figure 4. HUVEC were transiently transfected with pGloSensor™-20F cAMP Plasmid and stimulated for up to 30 min without or with 15 µM forskolin (18 hours after Nucleofection®). Stimulation and data collection at 26°C.

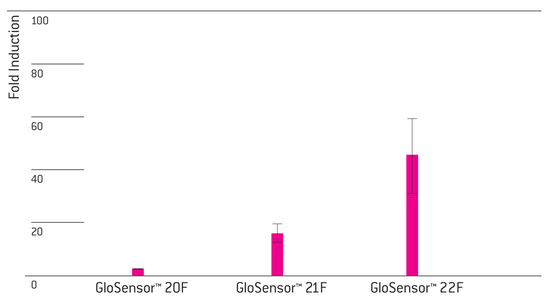

Figure 5. hMSC were transiently transfected with various pGloSensor™ Plasmids and stimulated for 15 minutes with 10 µM forskolin (18 hours after Nucleofection®). Stimulation and data collection at 26°C.

NFAT signaling in primary cells

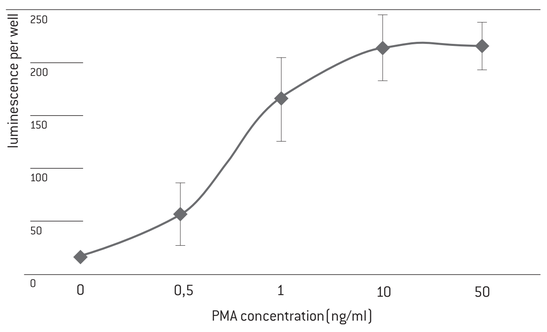

Figure 6. Dose-response to PMA/ionomycin of HMVEC-L transiently transfected with an NFAT-inducible luciferase construct.

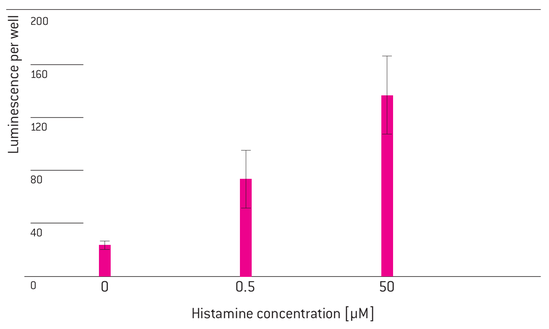

Figure 7. HUVEC were transiently transfected with an NFAT-inducible luciferase construct. Two hours after transfection, cells were stimulated with different histamine concentrations for 5 hours.

cAMP signaling in primary cells

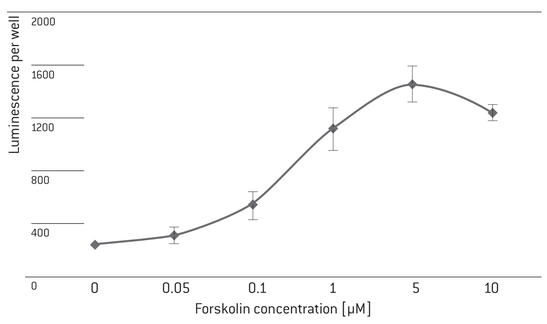

Figure 8. Dose-response to forskolin of HMVEC-L transiently transfected with a cAMP-inducible luciferase construct.

5. Conclusions

We showed that cAMP signaling can be reliably monitored in primary cells and stem cells after transient transfection of Promega's GloSensor™ using the Amaxa® Nucleofector® Technology. This concept can also be applied to analyzing activity of different signal transduction pathways in primary cells, as demonstrated here with CRE- and NFAT-RE-luciferase reporters. Our findings open new possibilities for employing primary cells in cell-based assays. Upscaling experiments to cryopreserve large numbers of transiently transfected cells either in vials or ready-to-use on multiwell plates are currently under way.