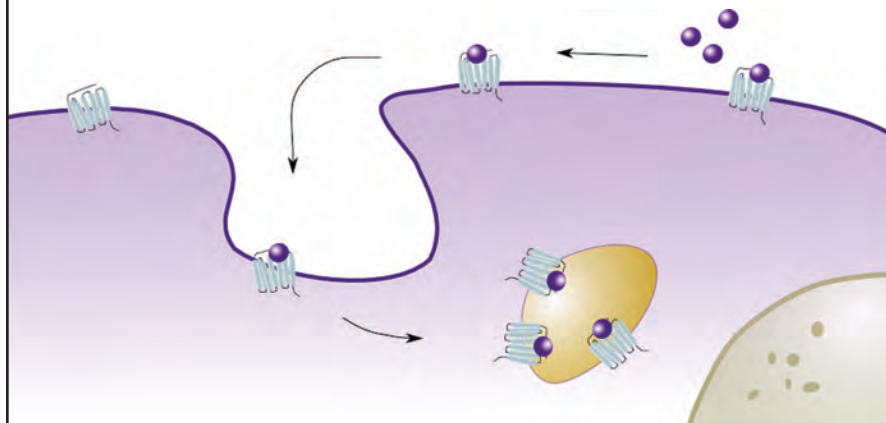


Image-based Quantification of Endothelin A Receptor (ET_AR) Translocation using the Opera



Key Features

- Automated confocal image acquisition of live cells using the Opera™ High Content Screening System
- Image-based detection of ET_AR translocation from membrane to cytoplasm
- Image analysis using the versatile Acapella™ Receptor Internalization script

Receptor Internalization

Background

Endothelins (ETs) are a class of peptide hormones with strong vasoactive properties [Bagnato & Natali, 2004]. ETs function by interacting with their G protein-coupled receptors (GPCRs). There are two known ET subtypes, ET_A and ET_B [Bremnes *et al.*, 2000]. The ET_A receptor (ET_AR) is localized on the plasma membrane until stimulated by endothelins such as endothelin-1 (ET-1), towards which ET_AR shows the highest binding affinity. ET-1 triggers ET_AR internalization into pericentriolar recycling endosomes. Cancer relevant processes such as proliferation and inhibition of apoptosis are regulated via the ET-1 / ET_AR pathway, making this a suitable target for cancer therapies.

The Opera High Content Screening (HCS) application described here focuses on the quantification of the translocation event mediated by ET-1.

Application

This assay monitors internalization of a fluorescently labeled ET_A Receptor. Unstimulated U2OS cells show membrane-bound fluorescence, whereas upon stimulation with the agonist ET-1, the GPCR internalizes into recycling endosomes. These can be detected and quantified as fluorescent "aggregates" in the vicinity of the cell's nucleus.

To generate a dose-response curve, ET-1 was serially diluted (0 – 100 nM) and was added to cells for 3 hr. After incubation at RT, cells were fixed (3 % formaldehyde) and the nuclei were stained with Hoechst 33342. The resulting images were acquired confocally on the Opera QEH5 with a high NA 20x water immersion objective.

The images were analyzed by applying the Acapella™ **Receptor Internalization** script. In this script the standard Nuclei and Cytoplasm Detection Library modules were used for the identification of nuclei and cells respectively. An expanded nuclear search region was used to look for the endosomes, since the internalized fluorescence signal is located in the vicinity of the nucleus (Figure 1). To prevent the search region from extending into adjacent cell areas, this was restricted to the detected cytoplasmic region of each cell.

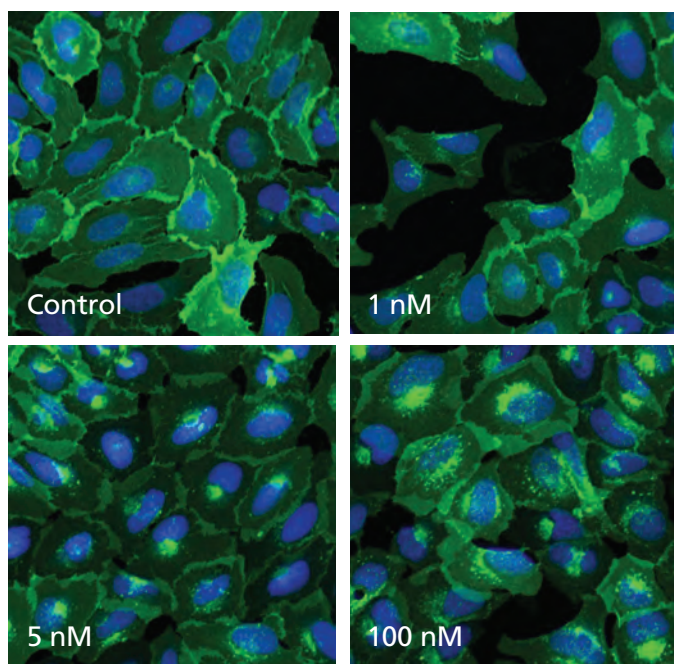


Figure 1. Images were captured using a 20x objective. A false color overlay of nuclear (blue) and ET_AR (green) fluorescence is shown. Translocation of ET_AR becomes apparent as the concentration of the agonist ET-1 is increased. Upon binding of ET-1, the receptor is internalized into pericentriolar recycling endosomes.

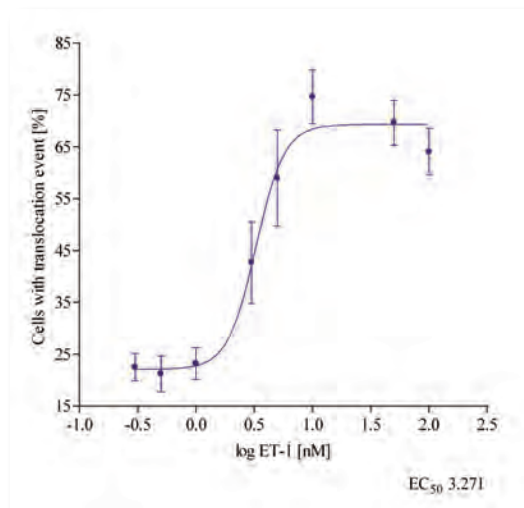


Figure 2. ET_AR activation in U2OS cells, measured upon addition of the ligand, ET-1. The graph shows the ET-1-generated dose-response curve, for measurements in 6 fields per well, after 3 hr incubation, resulting in an EC₅₀ of 3.2 nM and a Z' value of 0.69. N = 3

Conclusions

We have demonstrated a versatile GPCR internalization assay using the Opera platform in combination with the Acapella software, which is ready-to-use for high throughput compound screenings. Figure 2 shows the dose-dependent translocation of ET_AR. This numerical data was used to determine an EC₅₀ value of 3.2 nM for ET-1.

References

- Bagnato A, Natali PG (2004): Endothelin receptors as novel targets in tumor therapy. *Journal of Translational Medicine*, 2 (1), 16.
- Bremnes T, Paasche JD, Mehlum A, Sandberg C, Bremnes B, Attramadal H (2000): Regulation and Intracellular Trafficking Pathway of the Endothelin Receptors. *Journal of Biological Chemistry*, 275 (23), 17596-17604.

Authors

Simone Schick Tanz
Eleni Mumtsidu

PerkinElmer

Cellular Technologies Germany GmbH
Cellular Imaging & Analysis
Hamburg, DE