Correlation between label-free impedance analysis and Ca^{2+} fluorescence *in vitro* imaging

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There are several approaches for investigating and unraveling GPCR-dependent signaling pathways using cell-based assays. You can choose between label-based and label-free measurement methods. One of the more modern label-based fluorescence indicators is the popular and frequently used genetically encoded calcium indicator GCaMP [1], which can be utilized to measure intracellular Ca^{2+} levels. Since Ca^{2+} is a crucial second messenger involved in various cellular mechanisms and signaling pathways, the GCaMP sensor can be applied to investigate GPCR-dependent signaling cascades. In addition to label-based methods, label-free, non-invasive measurement methods can also be used to detect possible GPCR activation, such as by measuring impedance, also known as ECIS (electric cell-substrate impedance sensing). In the ECIS assay, any change in cell shape results in a change in the measured impedance [2]. Since GPCR activation can lead to a remodeling of the actin cytoskeleton [3], this label-free measurement method is also well-suited for investigating GPCR pharmacology. Although these two different measurement methods (GCaMP and ECIS) are often used independently, there are no scientific studies yet that use both assay approaches and investigate how and to what extent the results of these two methods correlate. If a strong correlation exists, combining Ca^{2+} fluorescence imaging with label-free impedance analysis has great potential for analyzing and deciphering GPCR signaling pathways more holistically, considering multiple perspectives. To investigate a possible correlation between the different approaches and their results, HEK293T cells were first transiently transfected with a plasmid of the Ca^{2+} sensor pN1-GCaMP6m-XC [4] and an empty vector, then incubated for 40–48 hours. The cells were subsequently preincubated with various inhibitors or chelators (FR900359 [Gq inhibitor], thapsigargin [SERCA inhibitor], BAPTA-AM [Ca^{2+} chelator], and Y-27632 [Rho kinase inhibitor]) at different concentrations before being stimulated with ATP to activate the GPCR signaling pathway. The measurement data of fluorescence and impedance signals were finally compared. In both label-based Ca^{2+} fluorescence imaging and label-free impedance analysis, the various inhibition approaches of the GPCR signaling pathway and changes in intracellular Ca^{2+} concentration after ATP stimulation are similarly well recognizable, making the results obtained highly consistent and reproducible. The combined use of Ca^{2+} fluorescence imaging and label-free impedance analysis thus offers enormous potential for analyzing and deciphering various GPCR signaling pathways more holistically, considering multiple perspectives.

References:

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