DSTC
Drug Safety Testing Center

Biochemical hERG channel trafficking assay using HEK/CHO cells

hERG タンパク質の生化学的定量解析

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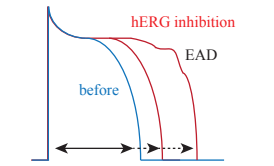
Introduction

hERG (human *ether-a-go-go*-related gene) potassium channels present in cardiac myocytes play a crucial role for repolarization of action potentials. It is known that drug-induced blockade of hERG channel activity is well correlated with QT interval prolongation due to delaying repolarization of action potentials, thus increasing life threatening arrthmogenic risk. The incidence of such cardiotoxicity is predicted to be elicited by acute and/or chronic blocking effects of compounds towards hERG. hERG expressed-HEK/CHO cells are widely used *in vitro* safety assay of candidate compounds. Recently, hERG trafficking assay has drawn an increasing attention in order to evaluate the chronic actions of the drug. The present study is aimed to investigate whether or not both HEK and CHO cell lines exhibit eqivalent sensitivity to known hERG trafficking inhibitors. To this end, we analyzed the expression levels of hERG proteins prepared from the two distinct cell lines after chronic treatment in the presence of the trafficking inhibitors, pentamidine and geldanamycin.

1 Blockade of hERG channels prolongs duration of actional potentials

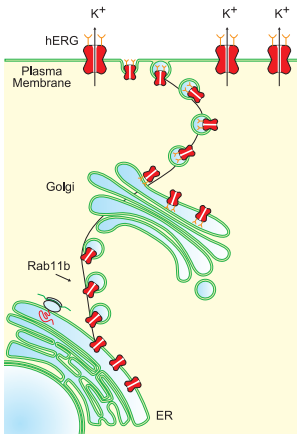


delayed repolarization of action potentials

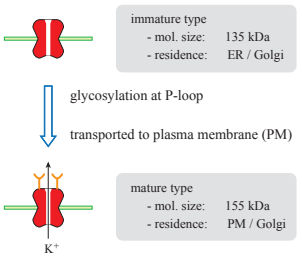
Acute blocking effects:
- inhibit hERG channel function in several minutes
- direct interaction with hERG channels
- can be evaluated with whole-cell patch clamp

Chronic blocking effects:
- minimal inhibition in the short-term, if any
- but prominent in several hours to a day
- interference with biogenesis and/or trafficking processes of hERG channels

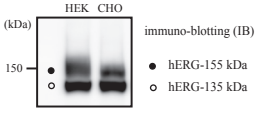
2 Trafficking processes of hERG



3 Functional maturation of hERG channel



4 Quatification of hERG channels

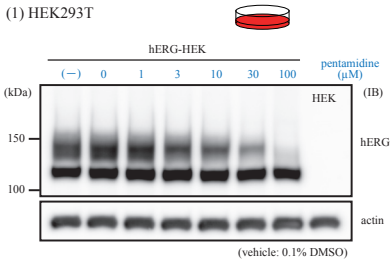


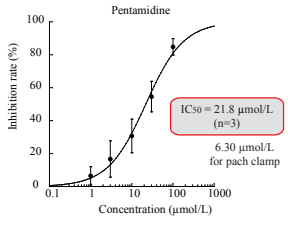
Chronic effects of pentamidine on hERG trafficking

24 hr incubation at different concentrations as indicated

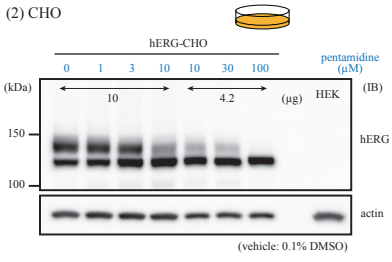
Pentamidine: endoplasmic reticulum (ER) export inhibitor

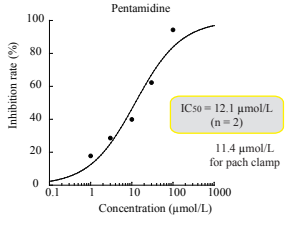
(1) HEK293T





(2) CHO



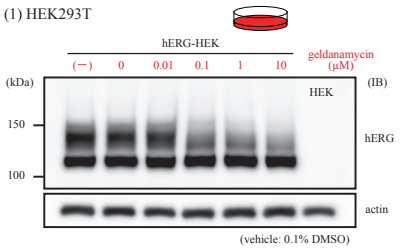


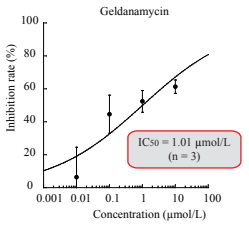
Chronic effects of geldanamycin on hERG trafficking

24 hr incubation at different concentrations as indicated

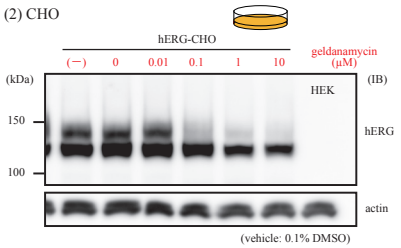
Geldanamycin: selective HSP90 inhibitor

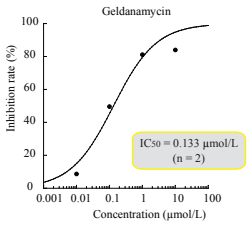
(1) HEK293T



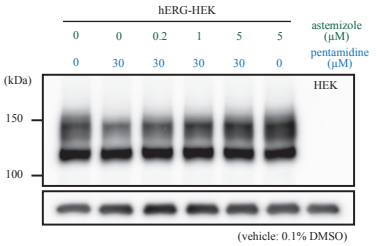


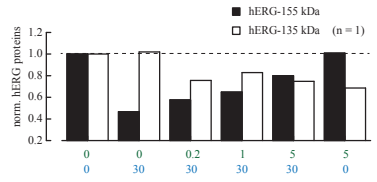
(2) CHO



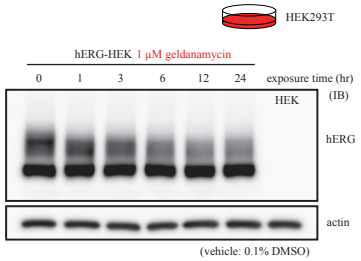


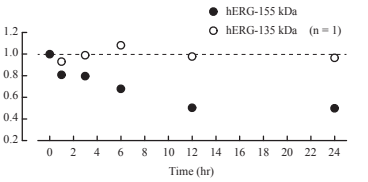
Rescue study





Time course study





Summary

In both cell lines, the trafficking inhibitors showed graded reduction of matured hERG proteins in a concentration-dependent manner, with slight difference of IC_{50}. The values from hERG-HEK cells were relatively higher than those from hERG-CHO cells (pentamidine: 1.8-fold; geldanamycin: 7.6-fold). These results showed a good correlation with those of hERG current measurement using whole-cell patch clamp technique, except for the blocking effect difference depending on cell-types. The results imply that blocking effects of compounds on hERG trafficking rely on the host species-derived cellular traits. After biosynthesized, hERG proteins get functionally matured and then are transported to cell surface through multiple intracellular processes. HEK and CHO cells possess distinct intracellular mechanisms for biogenesis/clearence of hERG channels including translation, post-translational modification, intracellular pool size, vesicular transport, quality of control, and so on, thereby possibly contributing to the difference of interfering effect on the hERG trafficking process. Also, different cytotoxicity sensitivity of compounds among cell lines (data not shown) could be in part attributable to the difference of IC_{50}.

(最後に)

hERG のタンパク質定量は、 成熟型 hERG のみならず未成熟型 hERG の量的変化を同時に捉えることができ、 生合成から細胞内輸送の過程において、 化合物によるトラフィッキング阻害機構に迫ることが考えられる。 また、トラフィッキング阻害の時間推移や回復、 化合物間の競合作用 (例 : レスキュー試験) を精査する試験としても有用である。 候補化合物の長期的安全性を確認する薬理試験としてお役立て頂きたい受託サービスです。