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Functional Abnormalities of HERG Mutations in Long QT Syndrome 2 (LQT2)

Masayasu HIRAOKA

Department of Cardiovascular Diseases Medical Research Institute Tokyo Medical and Dental University 1-5-45, Yushima, Bunkyo-ku, Tokyo 113−8510, Japan

The chromosome 7-linked long QT syndrome (LQT2) is caused by mutations in the *human ether-a-go-go-related gene* (*HERG*) that encodes the rapidly activating delayed rectifier K^+ current, I_{Kr}, in cardiac myocytes. Different types of mutations have been identified in various locations of HERG channel. One of the mechanisms for the loss of normal channel function is due to membrane trafficking of channel protein. The decreased channel function in some deletion mutants appears to be due to loss of coupling with wild type *HERG* to form the functional channel as the tetramer. Most of missense mutants with few exceptions could interact with wild type *HERG* to form functional tetramer and caused dominant negative suppression with co-injection with wild type *HERG* showing variable effects on current amplitude, voltage dependence, and kinetics of activation and inactivation. Two missense mutants at pore regions of HERG found in Japanese LQT2 (A614V and V630L) showed accentuated inward rectification due to a negative shift in steady-state inactivation and fast inactivation. One mutation in S4 region (R534C) produced a negative shift in current activation, indicating the S4 serving as the voltage sensor and accelerated deactivation. The C-terminus mutation, S818L, could not express the current by mutant alone and did not show dominant negative suppression with co-injection of equal amount of wild type cRNA. Co-injection of excess amount of mutant with wild type produced dominant negative suppression with a shift in voltage dependent activation. Therefore, multiple mechanisms are involved in different mutations and functional abnormality in LQT2. Further characterization with the interactions between various mutants in *HERG* and the regulatory subunits of the channels (*MiRP1* and *minK*) is to be clarified.

Key Words: I_{Kr} current, Inward rectification, Missense mutation, Trafficking defect, Dominant negative suppression

INTRODUCTION

The molecular genetics combined with electrophysiological technique have disclosed the pathogenesis of inherited cardiac arrhythmias, long QT syndrome (Vincent, 1998). In addition, the studies have made innumerable contribution to our understanding of functional and structural aspects of cardiac ion channels, which form normal cardiac repolarization.

Corresponding to: Masayasu HIRAOKA, Department of Cardiovascular Diseases Medical Research Institute Tokyo Medical and Dental University 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8510, Japan, (Tel) 81-3-5803-5829, (Fax) 81-3-5684-6295, (E-mail) hiraoka.card@mri.tmd.ac.jp

The *ether a-go-go-related gene (eag)* had originally been cloned from a *Drosophila* mutant displaying abnormal movements upon exposure to ether (Warmke et al, 1991) and subsequently, homologs of *eag* were cloned in mouse and rat. In human hippocampus, a related gene, the *human ether a-go-go-related gene* (*HERG*) was cloned (Warmke & Ganetzky, 1994). After the chromosome 7-linked long QT syndrome was found to be caused by HERG mutation (Curran et al, 1995) and *HERG* was discovered to encode the rapidly activating component of delayed rectifier K^+ current, I_{Kr}, in cardiac myocytes (Curran et al, 1995; Trudeau et al, 1995), this current has received extensive attention by the fields in physiology, pharmacology and channel biophysics. The HERG channel

displays voltage-dependent activation and inactivation. The activation and deactivation kinetics are rather slow compared to the inactivation time course which displays decreased current amplitudes on depolarization to voltages positive to around 0 mV, a unique feature of inward rectification (Curran et al, 1995; Trudeau et al, 1995; Smith et al, 1996). Upon repolarization to negative potential levels, the channel inactivation recovers quickly in spite of relatively slow deactivation, allowing the channel to return to the open state and, thus, developing a large outward tail current with time-dependent decay, which forms the rapid repolarization phase (phase 3) of cardiac action potential. A high sensitivity of the HERG/I_{Kr} current to external K^+ concentration can be explained by the K^+-dependence of inactivation kinetics (Curran et al, 1995; Trudeau et al, 1995; Wang et al, 1996; Yang & Roden, 1996). Furthermore, the HERG/I_{Kr} current is a main target of the certain class of antiarrhythmic agents to prolong action potential duration and other non-cardiac agents sharing the similar action (Sanguinetti & Jukiewicz, 1990; Vorperian et al, 1996; Yang & Roden, 1996; Drolet et al, 1998; Ficker et al, 1998; Drolet et al, 1999; Zhang et al, 1999). These drugs may sometimes induce unexpected side effects, drug-induced or acquired QT prolongation.

THE LOSS OF FUNCTIONS IN HERG MUTATIONS

Since the first description of HERG mutations in LQT2 (Curran et al, 1995), various mutations of different types have been identified at various locations of the channels (Fig. 1) (see Roden & Balser, 1999). Functional abnormalities of these mutations appear to be caused by multiple mechanisms. Some mutant channels are not generated HERG/I_{Kr} current in heterologous expression system. A major reason for this defect seems to be due to problem with trafficking of the protein to the membrane (Zhou et al, 1998; Furutani et al, 1999; Petrecca et al, 1999), while others can be detected the protein at cell surface, suggesting a defect in channel gating (Zhou et al, 1998). Recently, defective protein trafficking of a mutant (N470D) has been found to be temperature-dependent and the defect can be corrected by the treatment with the specific blockers of I_{Kr} current including E-4031 (Zhou et al, 1999). For the recapitulation of the functional HERG/I_{Kr} channel, the C-terminal domain seems to play an essential role (Kupershmidt et al, 1998; Zhou et al, 1998), but the detailed mechanism is still undefined.

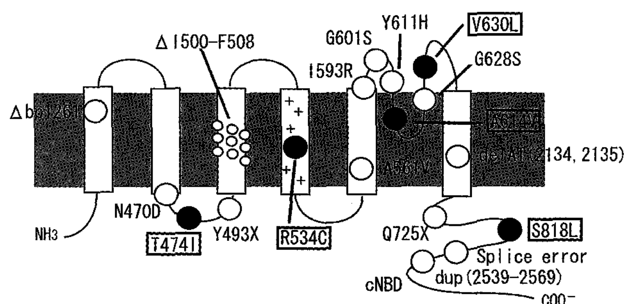

Fig. 1. Mutation sites of HERG in LQT2. There have been reported over 60 different types of mutations at numerous sites in HERG among LQT2 families in the world and some of representative mutations are indicated here.

For the functional characterization of HERG mutants, a common approach is to co-express wild type (WT) and mutant cDNAs in the expression system, in analogy with what is assumed to generally occur in a patient of Romano-Ward syndrome with a WT and mutant allele (Sanguinetti et al, 1996). Two deletion mutants (bp1261; a single base-pair deletion causing frameshift in S1, and I500-F508; nine amino acid deletion in S3) did not express the current when they were injected alone. When they were co-injected with WT, the amplitude and voltage dependence of expressed current were the same as those injected with WT alone, indicating no interaction between mutant and WT proteins to form functional tetramer. Three missense mutations (N470D in S2, A561V in S5 and G628G at pore region) produced variable effects on I_{Kr}. Injection of each mutant could or could not express the current. Co-injection with WT produced dominant negative suppression with variable degrees.

The mutations in the N-terminus of *HERG* result in abnormally fast deactivation, in which they cause the reduction of outward current upon repolarization to negative voltages and, thus, induce decreased tail current during phase 3 repolarization leading to prolonged QT intervals (Chen et al, 1999). Another mutation in N-terminus of HERG (bp 1261 mutation) results in a truncated protein that contains a subunit interaction domain and decreases channel expression (Li et al, 1997). Mutation at the pore selectivity

signature sequence from GFGN to GFGD (N629D) has recently been shown to exhibit a unique gain-of-function mechanism causing altered channel gating and loss of K^+ selectivity (Lees-Miller et al, 2000). This mutation produces markedly reduced outward tail current or induction of inward tail current upon repolarization, which forms the basis of QT prolongation.

THE MECHANISMS OF CHANNEL DYSFUNCTION IN HERG MUTATIONS FOUND IN JAPANESE LQT2 FAMILIES

We studied functional abnormalities found in HERG mutations found in Japanese LQT2 families (Tanaka et al, 1997). First, functional characterization of three mutations (T474I; cytoplasmic linker between S2 and S3, A614V and V630L; outer mouth of pore region) was done in heterologous expression system in *Xenopus* oocytes (Nakajima et al, 1998). Injection of each mutant alone did not express functional channel currents. Co-injection of WT with each mutant produced current with decreased amplitude showing dominant negative suppression in an order of T474I $<$ A614V $<$ V630L. Conductance curves showed increased inward-going rectification at plateau voltages with the most prominence in V630L and the least in T474I. Steady-state inactivation curve was shifted to negative direction by 20 mV in V630L and 7 mV in A614V, but no change was noted in T474I. Fast inactivation and recovery from inactivation in V630L, but not in T474I and A614V, became faster than those in WT. Therefore, shift in voltage-dependent inactivation and fast inactivation of HERG channel seems to be a novel mechanism for current suppression in LQT2.

The S4 region in *HERG* is assumed to be a voltage sensor as like other voltage-gated K^+ channels, but this issue has not been confirmed in this channel. Since a mutation in S4 region, R534C, was found in Japanese LQT2 family (Itoh et al, 1998), functional characterization of this mutation was expected to explore not only the mechanism of current suppression, but also the role played by S4 in *HERG* might be clarified. R534C mutant alone could express the HERG current with reduced amplitude and the expressed current showed a voltage shift in activation in negative direction, indicating the S4 actually serving as the voltage sensor. Co-injection of R534C and WT did not suppress the current amplitude, indicating that this mutation did not cause dominant negative suppression. In addition, the R534C current showed faster deactivation than that of WT current, which might be the reason for decreased outward current upon repolarization as the basis of QT prolongation (Nakajima et al, 1999). However, our computer simulation incorporating these functional abnormalities seen in R534C mutant could not reproduce action potential prolongation. Therefore, the actual reason to cause QT prolongation in the affected members of this LQT2 family remains to be clarified.

The C-terminus of *HERG* is not well characterized in its functional aspect except a defect of intracellular protein transport to the plasma membrane is indicated (Zhou et al, 1998). Recently a mutation in the C-terminus of HERG channel, S818L, has been reported (Berthet et al, 1999) and we also found the mutation in Japanese LQT2 family. So, we characterized the functional defect in S818L expressed in *Xenopus* oocytes (Nakajima et al, 2000). S818L alone could not express current and co-injection with equal amount of WT developed current without showing dominant negative suppression. However, when excess amounts of mutant cRNA (x3 ~ x10) were co-injected with WT (x1), expressed current was suppressed with concentration-dependent manner of S818L, exhibiting dominant negative effect. Furthermore, expressed current showed a negative shift in activation voltage and slowed activation and deactivation time course. These results indicate that mutant S818L can form the tetramer with WT to function as HERG channels, and the C-terminus of HERG may, in part, involve in activation kinetics.

THE ROLE OF THE REGULATORY SUBUNIT FOR THE HERG CHANNEL DYSFUNCTION

The heterologouly expressed HERG current roughly reproduces current similar to native I_{Kr}, but it differs in certain aspects, such as gating, single channel conductance, regulation by external K^+ and sensitivity to antiarrhythmic agents (Shibasaki, 1987; Sanguinetti et al, 1990; Sanguinetti et al, 1995; Trudeau et al, 1995; Spector et al, 1996; Yang et al, 1996; Wang et al, 1997). Recently, a *minK*-related peptide 1 (*MiRP1*) has been cloned, which forms stable assem-

blies with HERG (Abbott et al, 1999). Co-assembled *HERG* and *MiRP1* produce the current features quite similar to the native I_{Kr} than the one expressed by *HERG* alone, such as a positive shit of activation, decreased peak as well as single channel current, less sensitive to external K^+ and faster deactivation. The *MiRP1/HERG* channels exhibit biphasic block by E-4031 similar to the native I_{Kr} channels; namely, application of E-4031 shows a significant inhibition with an initial pulse and achieve equilibrium blockade slowly with repetitive pulsations, while HERG channels exhibit little first pulse block and slowly develoing inhibition with repetitive pulses. Furthermore, several mutants found in *MiRP1* produced the decreased currents due to positive shift in activation or faster deactivation. One mutant in *MiRP1* (Q9E) showed increased blockade by the macrolide antibiotics. These findings suggest that genetically based reduction in K^+ current remains silent clinically until combined with additional stressors and might be the basis of acquired long QT syndrome and ventricular arrhythmias. There have been indicated that *minK* might interact with *HERG* (Bianchi, 1998) and mutant *minK* altered the function of *HERG*. Therefore, the functional characterization of HERG mutants co-assembled with *MiRP1* or *minK* has to be further clarified.

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