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Review

Molecular pathogenesis of long QT syndrome type 2

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ABSTRACT

The molecular mechanisms underlying congenital long QT syndrome (LQTS) are now beginning to be understood. New insights into the etiology and therapeutic strategies are emerging from heterologous expression studies of LQTS-linked mutant proteins, as well as inducible pluripotent stem cell derived cardiomyocytes (iPSC-CMs) from LQTS patients. This review focuses on the major molecular mechanism that underlies LQTS type 2 (LQT2). LQT2 is caused by loss of function (LOF) mutations in *KCNH2* (also known as the *human Ether-à-go-go-Related Gene* or *hERG*). Most LQT2-linked mutations are missense mutations and functional studies suggest that ~90% of them disrupt the intracellular transport (trafficking) of *KCNH2*-encoded Kv11.1 proteins to the cell membrane. Trafficking deficient LQT2 mutations disrupt Kv11.1 protein folding and misfolded Kv11.1 proteins are retained in the endoplasmic reticulum (ER) until they are degraded in the ER associated degradation pathway (ERAD). This review focuses on the quality control mechanisms in the ER that contribute to the folding and ERAD of Kv11.1 proteins; the mechanism for ER export of Kv11.1 proteins in the secretory pathway; different subclasses of trafficking deficient LQT2 mutations; and strategies being developed to mitigate or correct trafficking deficient LQT2-related phenotypes.

Contents

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1. Introduction

1.1. Congenital long QT syndrome

Long QT syndrome (LQTS) is a condition characterized by delayed ventricular and atrial repolarization, prolongation of the QT interval on an electrocardiogram (ECG), and an increased risk for the potentially fatal tachyarrhythmia Torsades de Pointes [1]. The first description of congenital LQTS probably dates back to Dr. Friedrich Ludwig Meissner's account of a deaf schoolgirl who died suddenly while being publically scolded in 1856 (Fig. 1) (http://www.qtsyndrome.ch/history.html). A century later, autosomal dominant LQTS (Romano-Ward Syndrome) was described as the major variant and a phenotypic diagnostic score based on patient ECG characteristics, symptoms, and family history was developed [2]. In the decades that followed, genetic mutations in cardiac ion channel related genes, as well as an increasing number of drugs, have been recognized to cause LQTS. Additionally, the major monogenic causes of congenital LQTS were identified: *KCNQ1* (LQT1), *KCNH2* (LQT2), and *SCN5A* (LQT3) [3–9]. LQT1–LQT3 make up ~90% of the genetically confirmed cases, and each type has distinct triggers, penetrance, and responsiveness to therapy [10]. Genetic testing for LQTS is commercially available and a genotype positive test for an unequivocal pathogenic LQTS mutation influences important clinical decisions [11].

1.2. Kv11.1 and the long QT syndrome type 2

KCNH2 (also known as the *human Ether-à-go-go-Related Gene* or *hERG*) encodes the voltage-gated K^+ (Kv) channel α-subunit Kv11.1. Four Kv11.1 α-subunits co-assemble into a tetrameric ion channel that conducts the rapidly activating delayed rectifier K^+ current (I_{Kr}) in the heart [4,7]. Loss-of-function (LOF) *KCNH2* mutations decrease I_{Kr} in type 2 LQTS (LQT2) [7,12,13]. The extent by which I_{Kr} is impacted in LQT2 is mutation-specific. It ranges from a small reduction in I_{Kr} to a nearly complete loss in I_{Kr}. Haploinsufficient mutations results in a LOF of the affected allele, whereas dominant negative (DN) mutations cause a LOF in the affected allele and negatively impact the function of the Kv11.1 proteins encoded by the normal or wild type (WT) allele. The QT interval is a reflection of the ventricular action potential (AP) duration. For didactic purposes, we performed computational AP simulations to show the impact that a 30% or 70% reduction in I_{Kr} has on the steady-state AP duration at different cycle lengths (Fig. 2) [14]. The prolongation in the AP duration is much more severe for the simulations with the 70% reduction in I_{Kr}. Consistent with this result, DN LQT2 mutations located in the pore region are linked to an increased risk for arrhythmic events in patients [15].

The mechanisms by which *KCNH2* mutations cause a LOF is understood in terms of the biophysical components that underlie macroscopic current (I) [16]. By definition, I is a product of the number of channels expressed in the cell membrane (n), channel open probability (Po), and the amplitude of the single channel current (i), so that $I=(n)\times(\mathrm{Po})\times(i)$ [17]. A LOF in one of these biophysical components can be used to classify LQT2-linked Kv11.1 mutations. Class 1 mutations disrupt the synthesis/translation of Kv11.1 α-subunits (decrease n); Class 2 mutations reduce the intracellular transport or trafficking of Kv11.1 proteins to the cell membrane (decrease n); Class 3 mutations disrupt Kv11.1 channel gating (decrease Po); and Class 4 mutations negatively affect K^+ permeation (decrease i).

About 40% of LQT2-linked *KCNH2* mutations are nonsense mutations, frameshift mutations, insertions, deletions, duplications, or involve a splice site that inhibits Kv11.1 protein synthesis/translation by generating incomplete proteins or causing nonsense-mediated RNA decay (NMD) (class 1 mechanism) [18–20]. By provoking NMD, class 1 mutations are expected to cause haploinsufficiency. The remaining ~60% of LQT2 mutations are missense, where a single nucleotide change alters an amino acid codon to a different amino acid to cause a LOF by disrupting channel trafficking to the cell membrane (class 2 mechanism), gating (class 3 mechanism), and/or single channel current (class 4 mechanism) [16,20–27]. Over 150 suspected LQT2-causing missense mutations have been studied using heterologous expression systems and these studies demonstrate that ~90% of LQT2-linked missense mutations disrupt Kv11.1 channel function via a class 2 mechanism (Fig. 3) [20–28]. Class 2 LQT2 mutations decrease the folding efficiency of Kv11.1 proteins and increase their retention in the endoplasmic reticulum (ER) by cellular quality control mechanisms.

This review focuses on the molecular mechanisms of the class 2 LQT2 phenotype. We summarize several findings for the regulation of WT Kv11.1 protein trafficking early in the secretory pathway. We also summarize the findings of several studies that investigate trafficking deficient LQT2-causing mutations.

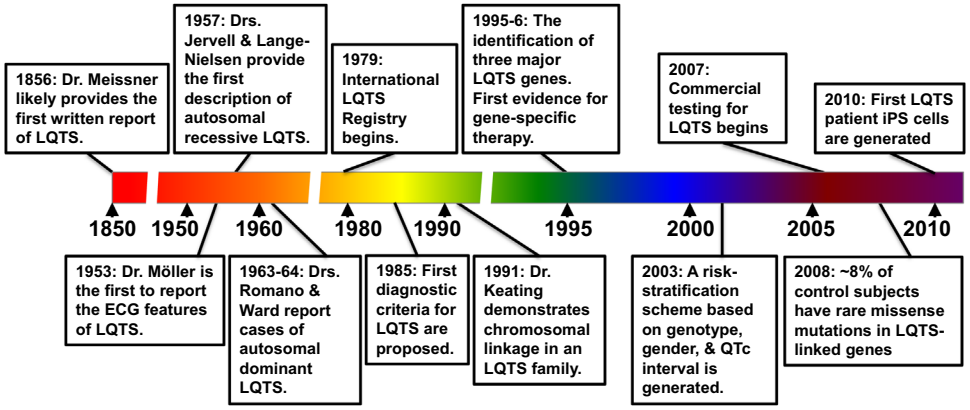

Fig. 1. A historical timeline of clinically relevant events in the study and management of congenital LQTS.

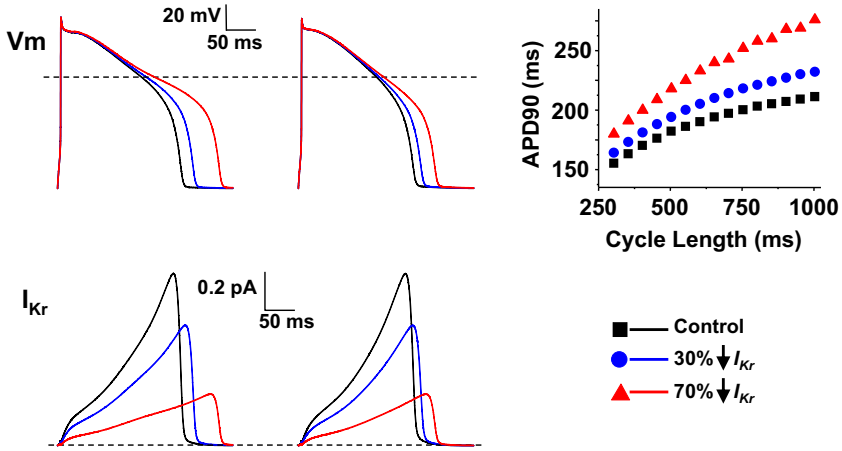

Fig. 2. Mutation-specific differences in I_{Kr} differentially affect ventricular APs. Computational simulations of the membrane voltage (V_m) results, using the Soltis–Saucerman rabbit ventricular myocyte AP model, are illustrated for normal conditions (37 °C and 5.4 mM extracellular [K^+], black traces) as well as following reduction in I_{Kr} by 30% (blue traces) or by 70% (red traces). Dashed lines represent 0 mV or 0 pA levels in V_m and I_{Kr} recordings, respectively. The duration of the steady-state ventricular AP at 90% repolarization (APD90) is plotted as a function of cycle length to predict the absolute changes at different heart rates.

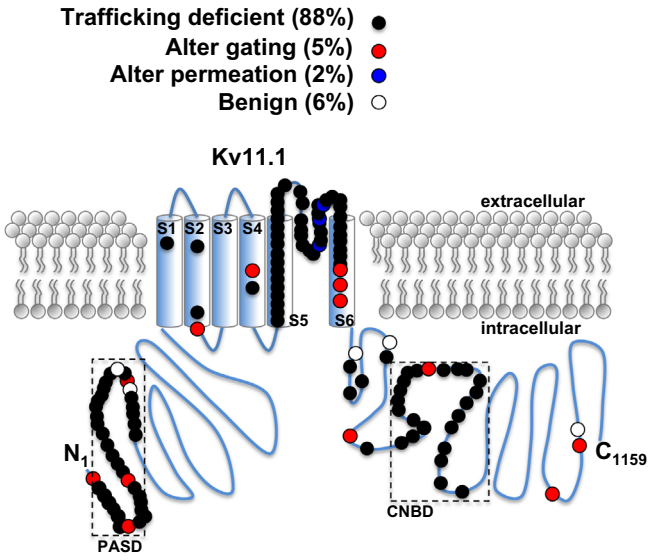

Fig. 3. Most LOF LQT2-linked missense mutations are trafficking deficient. The diagram illustrates a Kv11.1a α-subunit with an intracellular NH_2 terminus (N_1), six transmembrane segments (S1–S6), and the COOH terminus (C_{1159}). The relative locations of different LQT2 Kv11.1a missense mutations are shown. Mutations that primarily disrupt Kv11.1a trafficking, gating, and ion permeation are shown by black, red, and blue circles, respectively. Functionally normal variants are shown by white circles. Most of the trafficking deficient mutations are located in the Per-Arnt-Sim domain (PASD) in the N-terminus, pore domain (S5–S6), or the cyclic nucleotide-binding domain (CNBD) in the COOH-terminus. Dashed boxes indicate the location of PASD and CNBD domains. See the study by Anderson and colleagues (2014) for specific details [28].

2. Kv11.1 biogenesis and ER export

2.1. K^+ channel biogenesis

A single Kv α-subunit contains cytosolic amino (NH_2) and carboxy (COOH) termini and six α-helical transmembrane segments (S1–S6; Fig. 3) [17]. Electron density maps of crystalized Kv channels suggest that each α-subunit has a voltage-sensor domain formed by S1–S4 and a pore domain formed by S5 and S6 [29]. The sequence of events during Kv channel biogenesis (based on previous findings investigating Shaker K^+ channels) consists of the following steps: (1) membrane insertion and asparagine-linked (N-linked) "core" glycosylation, (2) oligomerization of α- and auxiliary subunits, (3) formation of the voltage-sensor and pore domains, and (4) juxtapositioning of adjacent amino (NH_2) and carboxy (COOH) termini [30]. A series of elegant studies demonstrate that Kv α-subunit secondary structures, including the tetramerization domain and transmembrane segments of the voltage sensor, begin to form inside the ribosomal tunnel during translation [31–36]. Even the S3–S4 hairpin "paddle" of the voltage-sensor forms near the exit of the ribosome tunnel [35]. These preformed secondary structures probably influence subsequent tertiary folding, timing and efficiency of membrane insertion events, and subunit co-assembly [32,36]. Therefore, it is not surprising that a single amino acid missense mutation can increase the probability of channel protein misfolding. Consistent with this concept, most class 2 LQT2 mutations localize to the highly structured regions in the Kv11.1a α-subunit, including the Per-Arnt-Sim domain (PASD) in the NH_2 terminus, the pore domain, and the cyclic nucleotide-binding domain (CNBD) in the COOH terminus (Fig. 3).

2.2. Kv11.1: assembly required

The I_{Kr} channel is a tetramer of *KCNH2* gene products and the *KCNE2* encoded MiRP1 auxiliary subunit. MiRP1 is a single transmembrane spanning peptide that modifies Kv11.1 channel gating and associates with several other ion channels in the heart [37,38]. Most studies that investigate drug block of I_{Kr} or LQT2 mutations focus on the full-length, 15-exon, 1159 amino acid Kv11.1a α-subunit (*hERG1a* or *KCNH2a*; Fig. 3). A second *KCNH2* gene product, the Kv11.1b α-subunit, is generated from an alternative start site on the 5' exon that reduces the protein size by deleting exons 1–5 (*hERG*1b or *KCNH2*b) [39]. Alternative splicing and polyadenylation of pre-mRNA can generate truncated versions of Kv11.1a and Kv11.1b transcripts (Kv11.1a-USO or Kv11.1b-USO) but these gene products are not functional [40,41]. Kv11.1a is the key functional α-subunit because Kv11.1b proteins are retained in the endoplasmic reticulum (ER) unless they heterotetramerize with Kv11.1a proteins [42].

2.3. Chaperones facilitate Kv11.1a folding and degradation

The ER is a reticular network that extends from the nucleus to the cell periphery. It contains a variety of domains and compartments that participate in the biosynthesis of proteins and lipids, Ca^{2+} storage, apoptosis signaling, cellular quality control, ER-associated degradation (ERAD), and ER export. The environment in the ER optimizes nascent protein folding, assembly, and

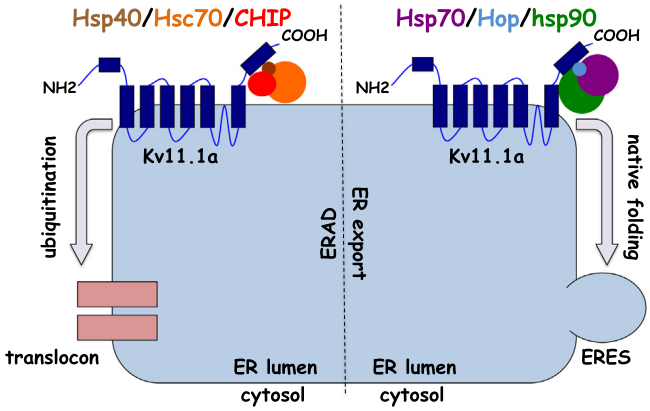

Fig. 4. A simplified illustration of the regulation of Kv11.1a protein folding and ERAD by cytosolic chaperones. Several studies suggest that Hsp40 activates Hsc70 and CHIP to facilitate ubiquitination/ERAD of Kv11.1a protein (left). Ubiquitinated Kv11.1a protein is transported into the cytosol via the translocon, where it is degraded by the proteasome. In contrast, Hsp70, HOP, and Hsp90 play a reciprocal role promoting native folding of Kv11.1a proteins and export them via transitional ER elements called ER exit sites (ERES, right).

maturation, while the ER quality control system prevents the trafficking of misfolded or incompletely assembled proteins to their target membranes [43]. Some proteins fold efficiently into their correct or "native" configuration but others do not. Molecular chaperone proteins in the ER or cytoplasm interact with nascent proteins to facilitate the folding process or target misfolded proteins in the ERAD pathway (Fig. 4).

Several different chaperone and co-chaperone proteins that interact with Kv11.1a proteins have been identified. They include the ER lectin chaperone calnexin, the ER transmembrane chaperone 38-kDa FK506-binding protein FKBP8 (FKBP38), cytosolic heat shock proteins 40, 70, and 90 (Hsp40, Hsp70, and Hsp90), the cytosolic heat shock cognate protein 70 (Hsc70), the cytosolic co-chaperone Hsp-organizing protein (HOP), the BCL-associated athanogene 2 (BAG-2), and the E3-ubiquitin ligase CHIP [44–46]. Ficker and colleagues (2002) were the first to demonstrate that Hsp90 is crucial for the maturation and trafficking of Kv11.1a channels to the cell membrane [44]. Unlike Hsp70 or Hsc70, which bind to most newly synthesized proteins, Hsp90 facilitates the folding for only a subset of specific proteins. Pharmacological inhibition of Hsp90 increases the ER retention and ERAD of Kv11.1a proteins. Iwai and colleagues (2013) show that Hsp90 expression increases the stability of Kv11.1a proteins by decreasing CHIP-mediated degradation in the ERAD pathway (Fig. 4, right) [47].

The Hsp90 co-chaperone FKBP38 is also important for Kv11.1a channel protein stability. Walker and colleagues (2008) found that knocking down FKBP38 expression levels negatively affects Kv11.1a channel protein trafficking early in the secretory pathway [45]. In another study, Walker and colleagues (2010) show that the expression of different Hsp40 co-chaperones activate Hsc70 to destabilize Kv11.1a proteins by increasing CHIP-mediated ERAD (Fig. 4; left) [48]. Li and colleagues (2011) found that expression of Hsp70 exerts the opposite effect of Hsc70 and increases Kv11.1a protein stability [43]. Based on these studies, a simplified model of the cytosolic chaperone/co-chaperone network that regulates the stability and trafficking of Kv11.1a proteins is provided in Fig. 4. Hsp40/Hsc70/CHIP facilitate the ubiquitination and ERAD of Kv11.1a proteins, and Kv11.1a proteins are transported to the cytosol through the translocon and then degraded by the proteasome. Alternatively, Hsp70 couples to Hsp90 (presumably via HOP) to prevent the ERAD of Kv11.1a proteins by CHIP and promote their native folding (Fig. 4, right).

Calnexin plays an important role in the biogenesis and quality control of N-linked glycoproteins [49]. It interacts with glycan moieties of newly synthesized glycoproteins until they are properly folded. If the glycoproteins fail to fold correctly, calnexin in conjunction with calreticulin can lead to their retention in the ER. Although the Kv11.1a α-subunit is synthesized in the ER as a ~135 kDa N-linked glycoprotein, the role that calnexin/calreticulin have in the ER retention of Kv11.1a is not clear [24,50]. N-linked glycosylation was initially thought to be obligatory for the ER export of Kv11.1a [51], but more recent studies show glycosylation-deficient Kv11.1a channels readily traffic to the cell membrane [52].

2.4. ER export

Once a protein achieves its native configuration, it is exported from the ER in transitional ER elements called ER exit sites (ERES). The small GTPase SAR1 initiates the polymerization of coat-associated protein II (COPII)-associated proteins at ERES [53,54]. As COPII transport vesicles pinch off of the ERES, GTPase-activating proteins (GAPs) hydrolyze GTP to GDP and SAR1 dissociates from the vesicle and the COPII vesicles mediate the trafficking of cargo proteins to the ER Golgi Intermediate Compartment (ERGIC). Kv11.1a proteins traffic out of the ER in COPII vesicles, because expressing a dominant-negative mutant of SAR1 prevents the ER export of Kv11.1a proteins to the Golgi apparatus and cell membrane [55].

In general, incompletely or misfolded proteins are retained in the ER because they are excluded from ERES. This is because incompletely or misfolded proteins interact with molecules in the other parts of the ER to prevent them from entering ERES and/or adaptor proteins in ERES selectively recruit only correctly folded proteins to ERES for export in COPII vesicles [49]. Consistent with the latter, Roti and colleagues identified a potential Kv11.1a adaptor protein, the Golgin-associated vesicular transport GM130 [56]. Their data suggest that GM130 interacts with the COOH-terminus of Kv11.1a and participates in the tethering and/or targeting of Kv11.1a-containing vesicles within the Golgi apparatus.

Once Kv11.1a proteins traffic to the Golgi apparatus, they are terminally glycosylated to a ~155 kDa glycoprotein [50]. The majority of the 155 kDa Kv11.1a proteins in the cell reside in the cell membrane because they are susceptible to extracellular protease digestion [57]. Many studies infer the trafficking efficiency of Kv11.1a or identify class 2 LQT2 mutations by using western blot analysis to quantify the relative amount of the immature (135 kDa) and mature (155 kDa) Kv11.1 proteins (Fig. 5).

3. Molecular mechanism of class 2 LQT2 mutations

3.1. Class 2 LQT2 mutations

Class 2 LQT2-linked mutations decrease the folding efficiency of Kv11.1a proteins and increase their degradation in the ERAD pathway [44,58]. Some class 2 LQT2 proteins also activate ER stress pathways [59]. Not surprisingly, cell culture conditions that stabilize or facilitate the native folding of misfolded proteins can increase the trafficking and functional expression of most class 2 LQT2 proteins to the cell membrane (Fig. 5) [60,61]. Zhou and colleagues (1999) were the first to show that culturing cells at lower incubation temperature (26 °C) or in drugs that bind to Kv11.1a proteins and block I_{Kr} increase the cell membrane and functional expression of certain class 2 LQT2 proteins (temperature or pharmacological correction, respectively; Fig. 5) [60]. Subsequent studies show that the trafficking deficient phenotypes for many different class 2 LQT2 proteins undergo temperature and/or pharmacological correction [28,62].

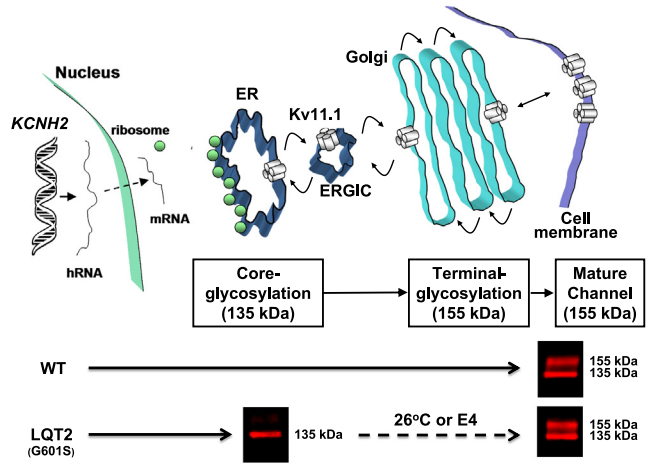

Fig. 5. A diagram of the Kv11.1 channel trafficking pathway. *KCNH2* is transcribed and spliced to mRNA, which is then translated in the ER as a core glycosylated Kv11.1a α-subunit with a molecular mass of 135 kDa. It undergoes terminal glycosylation in the Golgi apparatus and its molecular mass increases to 155 kDa. Shown are representative Western blots for cells expressing WT Kv11.1a proteins or the class 2 LQT2 proteins harboring the G601S mutation. In control conditions, the immunoblot of cells expressing WT Kv11.1a proteins contains both immature and mature forms (top solid arrow), whereas cells expressing the Kv11.1a proteins with the G601S mutation generate only the immature form (bottom solid arrow). Incubating cells at 26 $\bar{7}$C or in the I_{Kr} blocker E-4031 (E4) overnight increases the mature form in cells expressing the Kv11.1 proteins with the G601S mutation (dashed arrow).

3.2. Temperature correction

Initial studies of different LQT2 proteins were done using heterologous expression in *Xenopus* oocytes [20]. These studies primarily focus on Kv11.1 channel gating and DN phenotypes [20,23,63]. However, Furutani and colleagues [61] found that the LQT2 mutation Gly601Ser (G601S) exhibits a large difference in the LOF phenotype when expressed in oocytes vs. mammalian cells. The reason for this discrepancy was unclear, but the authors speculate that it was secondary to the differences in the incubation temperatures of the different cells types. Zhou and colleagues (1999) found that lowering the incubation temperature of mammalian cells from 37 °C to 26 °C increases the terminal glycosylation and functional expression of the class 2 LQT2 mutant protein N470D [64]. Recently, Anderson and colleagues (2014) [28] performed a comprehensive analysis of LQT2-linked missense mutations and demonstrated a clear domain-dependence to temperature correction. Western blot analysis of cells expressing different class 2 LQT2 proteins show that the protein trafficking for 80% (40/49) of the mutations in the PASD, 20% (12/60) of the mutations in the pore domain, and 60% (15/24) of mutations in the CNBD increases at lower incubation temperatures [28].

3.3. Pharmacological correction

Zhou and colleagues (1999) also showed that culturing cells in I_{Kr} blockers (i.e. the class III anti-arrhythmic E-4031) improves the trafficking of some class 2 LQT2 proteins [60]. The amino acid residues Y652 and F656 in S6 of the Kv11.1a α-subunit pore domain are critical for the high-affinity block of I_{Kr} [65,66]. Ficker and colleagues (2002) found that pharmacological correction of class 2 LQT2 proteins is strongly reduced when F656 was mutated into a more hydrophilic cysteine [67]. Conversely, engineering second site mutations at Y652 mitigates the trafficking deficient class 2 LQT2 phenotype for several class 2 LQT2 proteins [68]. Taken together, these results demonstrate the significance of hydrophobic drug–pore interactions for increasing the trafficking of class 2 LQT2 proteins and I_{Kr} block. Since these original reports, dozens of different trafficking deficient LQT2 proteins are shown to undergo pharmacological correction with I_{Kr} blockers [28]. Anderson and colleagues (2014) found that pharmacological correction also depends on the domain the mutation is located. Western blot analyses of cells expressing class 2 LQT2 proteins show that incubating cells in E-4031 causes pharmacological correction for 47% (23/49) of the mutations in the PASD, 33% (20/60) of the mutations in the pore domain, and 21% (5/24) of the mutations in the CNBD [28].

It remains to be seen if the drug–channel interactions that improve the trafficking of LQT2 proteins can be dissociated from the interactions that mediate drug block of I_{Kr}. This would have important implications as a potential therapeutic approach to improve mutant Kv11.1a protein trafficking in many LQT2 patients. There are currently several drugs that appear to improve LQT2 protein trafficking at drug concentrations much lower than the drug concentration needed to block I_{Kr}. However, it is not clear whether these drugs normalize the trafficking of LQT2 proteins by binding to the intracellular vestibule or via other unidentified mechanisms [69,70]. These studies identify the potential for further development and application of pharmacological correction as a novel therapeutic approach in some patients/families with LQT2.

3.4. Subclasses of class 2 mutations

It should be noted that the trafficking or function of WT Kv11.1a proteins is not improved by incubation at lower temperatures or in the presence of drugs that cause temperature or pharmacological correction of class 2 LQT2 proteins [60,62]. This suggests that temperature and pharmacological correction specifically compensate for mutation-specific changes in the native folding of Kv11.1a proteins rather than simply increasing overall Kv11.1a protein stability [68]. This is consistent with the mutation- and domain-specific patterns of temperature and pharmacological correction [28,71]. For example, several class 2 LQT2 mutations located in the PASD and CNBD only undergo temperature correction, whereas none of the class 2 LQT2 mutations in the pore domain only undergo temperature correction. All of the pore domain mutations that undergo temperature correction also undergo pharmacological correction. Additionally, several class 2 LQT2 mutations in the pore domain only undergo pharmacological correction, but none of the class 2 LQT2 mutations in the PASD and CNBD show pharmacological correction only. All of the mutations in the PASD or CNBD that undergo pharmacological correction also undergo temperature correction. These domain-specific differences in correction suggest that temperature correction facilitates the folding of Kv11.1a proteins at multiple steps in the biogenic pathway (i.e. assembly, formation of the voltage-sensor/pore domains, increasing the proximity of the NH_2 and COOH termini, etc.), whereas pharmacological correction facilitates folding of Kv11.1a intermediates late in the biogenic pathway (e.g., after the formation of the pore domain).

3.5. Chaperones interact with Kv11.1 channels affected by class 2 LQT2 mutations

Hsp90 is critical for folding and trafficking of WT Kv11.1a proteins. Therefore, it is not surprising that Hsp90 influences ER retention and ERAD of Kv11.1a proteins affected by class 2 LQT2 mutations. Several different class 2 LQT2 proteins remain tightly associated with Hsp90 and Hsp70 in the ER [44]. Temperature and pharmacological correction cause class 2 LQT2 channel–chaperone complexes to dissociate from one another. Iwai and colleagues (2013) found that Hsp90 overexpression decreases the binding of

CHIP to the COOH terminus for several class 2 LQT2 proteins and increases the functionality of class 2 LQT2 mutant proteins that harbor a frameshift mutation in the COOH-terminus [47]. Overexpression of Hsp90 or the Hsp90 co-chaperone FKBP38 also increases the trafficking for several other class 2 LQT2 proteins [45,72]. Together the data suggest that Hsp90 might limit ERAD, stabilize Kv11.1a proteins, and facilitate the folding for certain class 2 LQT2 proteins.

The roles that Hsp40, Hsc70, and Hsp70 have on the trafficking of class 2 LQT2 proteins are less clear. Although expression of Hsp40 decreases the trafficking of WT Kv11.1a proteins in cells incubated at physiological temperature, expression of Hsp40 does not negatively affect WT Kv11.1a protein trafficking at lower incubation temperatures [48]. However, the expression of Hsp40 inhibits the temperature correction of class 2 LQT2 proteins with the G601S mutation. This means that subtle structural differences likely still exist between WT and some class 2 LQT2 proteins, even when cells are incubated at temperatures that promote correction. Li and colleagues (2011) found that the degree of Hsc70 association with different class 2 LQT2 proteins depends on the topological location of the mutation [43]. The level of Hsc70-associated is higher for class 2 LQT2 mutations located in cytosolic domains (i.e. NH_2 and COOH termini). Additionally, they found that heat shocking the cells (to increase Hsp70 expression) preferentially promotes the maturation of class 2 LQT2 proteins. These data raise the possibility that limiting the association of Hsc70 with Kv11.1a might have therapeutic potential for class 2 LQT2 mutations in cytosolic domains.

3.6. Sequestration of proteins affected by class 2 LQT2 mutations

Immunocytochemistry studies show that class 2 LQT2 channels are selectively stored in a microtubule-dependent ER compartment that colocalizes with the transitional ER marker BAP31 (but not the perinuclear ER marker calnexin; the ERAD domain marker Derlin; the ERES marker Sec31; or the ERGIC marker ERGIC-53) [73,74]. Studies show that incubating cells in brefeldin A (bfa, a fungal metabolite that inhibits the retrograde transport of proteins to the ER) and the microtubule depolymerizing agent nocodazole blocks ER export and causes export cargo to accumulate in large spherical or dilated ERES easily visualized using microscopy [73,75]. Treating cells expressing class 2 LQT2 proteins in bfa and nocodazole confirms that they are excluded from the ERES. Interestingly, Roti and colleagues (2002) show that several class 2 LQT2 proteins do not to bind to GM130 [56]. These data provide a working model whereby some class 2 LQT2 proteins are stored in a transitional ER compartment and excluded from ERES because they fail to associate with the putative Kv11.1a adapter GM130.

Studies show that pharmacological correction directly increases the trafficking of class 2 LQT2 proteins stored in the transitional ER compartment [60,74]. These findings are surprising because they suggest that a steady-state pool of class 2 LQT2 proteins exist in the ER that are not yet targeted for ERAD. The implications are that cells can be briefly incubated in I_{Kr} blockers to increase the functional expression of class 2 LQT2 proteins sequestered in the ER. Once the class 2 LQT2 proteins traffic to the membrane, they remain functionally active for several hours after the drug has been washed out [74]. These data provide an important proof of principle that I_{Kr} blockers can be acutely administered to cause pharmacological correction of class 2 LQT2 proteins and increase I_{Kr}.

3.7. Patient-specific cellular models for class 2 LQT2

The ability to generate inducible pluripotent stem cell derived cardiomyocytes (iPSC-CMs) offers a new cellular platform for identifying patient-specific molecular mechanisms of LQT2 and developing personalized drug therapy [76]. Itzhaki and colleagues (2011) were first to obtain an iPSC line from a patient with the class 2 LQT2 missense mutation A614V [77]. The LQT2-patient derived iPSC-CMs show a significant prolongation of the AP, smaller I_{Kr}, marked arrhythmogenicity, early afterdepolarizations (EADs), and triggered arrhythmias [77]. Importantly, the authors were able to use the cells to evaluate the potency of several pharmacological agents that worsened or mitigated the cellular LQT2 phenotype.

Bellin and colleagues (2013) developed patient-derived iPSC-CMs from a patient with the class 2 LQT2 mutation N996I [78]. They used targeted gene correction of the N996I allele to restore I_{Kr} density and normalize AP duration in this iPSC-CM line. Moreover, introducing the same mutation into human embryonic stem cells (hESCs) result in the development an electrophysiological phenotype similar to the one detected in iPSC-CMs from the patient. These data demonstrate that genetic modification of iPSC-CMs and hESC can clearly delineate the pathogenic cause underlying a patient's disease phenotype with a suspected LQTS-causing mutation.

Matsa and colleagues studied iPSC-CMs derived from a patient with the DN class 2 LQT2 mutation A561T [79]. They confirmed the class 2 phenotype in iPSC-CMs and used allele-specific RNA interference (RNAi) to selectively degrade mutant mRNA while leaving WT mRNA intact. Treating the LQT2 iPSC-CMs with the mutation-specific siRNA normalized the AP duration, increased I_{Kr}, and decreased the frequency EADs. More recently, Mehta and colleagues (2014) studied iPSC-CMs derived from a patient with the related class 2 LQT2 mutation A561V [80]. They examined the relative expression of the A561V and WT alleles and found that the A561V allele is expressed at a higher level than the WT allele. Furthermore, they found that several quality control genes including cysteine proteases, Hsp90, Hsp70, and ubiquitin are up regulated in LQT2 iPSC-CMs compared to their levels in control cells. In an attempt to correct the class 2 LQT2 phenotype, the investigators incubated LQT2 iPSC-CMs with ALLN (*N*-[*N*-(*N*-acetyl-L-leucyl)-L-leucyl]-L-norleucine), a calpain/proteasome inhibitor. ALLN incubation decreases the expression of several of the calpains, Hsp70, and ubiquitin genes, but increases the expression of Hsp90. Importantly, ALLN incubation increases the trafficking of the Kv11.1a proteins, increases I_{Kr}, and decreases the AP duration in the LQT2 iPSC-CMs.

4. Conclusions

4.1. Summary

LQT2 is the second most common type of Romano Ward LQTS. It is caused by mutations in *KCNH2* that primarily decrease the number of I_{Kr} channels in the cell membrane by disrupting channel synthesis (class 1 mechanism) or their trafficking (class 2 mechanism). Functional I_{Kr} channels in cardiac cells are generated by the co-assembly of Kv11.1a/Kv11.1b α-subunits and the auxiliary K^+ channel subunit MiRP. The folding and ER export of Kv11.1a is dependent on Hsp90, and the stability/ERAD of Kv11.1a is regulated by its interactions with Hsp40, Hsp/c70 and the E3 ligase CHIP. Studies suggest that Kv11.1a is exported from the ER in COPII vesicles and its trafficking early in the secretory pathway is influenced by the putative adaptor protein GM130. Class 2 LQT2 mutations primarily occur in the PASD, pore domain, or CNBD. Incubating cells at reduced temperatures or in drugs that bind Kv11.1a and block I_{Kr} can correct the trafficking for many different class 2 LQT2 proteins. Some of these mutated proteins are

excluded from ERES and become sequestered in the transitional ER compartment prior to being degraded via ERAD. The class 2 LQT2 mechanism has been verified in iPSC-CMs from a small number of different LQT2 patients. These studies show that targeted gene correction of the LQT2 mutation, siRNA knock down of the mutant mRNA, or incubating cells in drugs that inhibit calpain/proteasome degradation mitigates the LQT2 patient iPSC-CM cellular phenotypes.

4.2. *In memoriam of Dr. Eckhard Ficker*

Dr. Eckhard Ficker, one of the pioneers of Kv11.1 channel studies, passed away suddenly in 2013. He was the first to describe subclasses of class 2 LQT2 mutations [71], the role of Kv11.1a binding domain for pharmacological correction [67], and the regulation of Kv11.1a trafficking by Hsp90/Hsp70 [44]. He made several other seminal discoveries related to Kv11.1a channels [81–88]. Eckhard's work was always innovative and he was very helpful to many investigators. He will be sadly missed.

Conflict of interest

All authors declare no conflict of interest related to this study.

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