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REVIEW
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Molecular Insights Into the Gating Kinetics of the Cardiac hERG Channel, Illuminated by Structure and Molecular Dynamics

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The rapidly activating delayed rectifier K^+ current generated by the cardiac hERG potassium channel encoded by *KCNH2* is the most important reserve current for cardiac repolarization. The unique inward rectification characteristics of the hERG channel depend on the gating regulation, which involves crucial structural domains and key single amino acid residues in the full-length hERG channel. Identifying critical molecules involved in the regulation of gating kinetics for the hERG channel requires high-resolution structures and molecular dynamics simulation models. Based on the latest progress in hERG structure and molecular dynamics simulation research, summarizing the molecules involved in the changes in the channel state helps to elucidate the unique gating characteristics of the channel and the reason for its high affinity to cardiotoxic drugs. In this review, we aim to summarize the significant advances in understanding the voltage gating regulation of the hERG channel based on its structure obtained from cryo-electron microscopy and computer simulations, which reveal the critical roles of several specific structural domains and amino acid residues.

Keywords: HERG potassium channel, cryo-electron microscopy structure, molecular dynamics simulations, voltage gating regulation, molecular mechanisms

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INTRODUCTION

Since the identification of the *KCNH2* (hERG) gene in 1994, a multitude of studies have gradually revealed the significant role of the fast component of the inward delayed rectifier potassium current (I_{Kr}) generated by the hERG potassium channel in the formation of the complete action potential (Curran et al., 1995; Perry et al., 2015). Following the identification of another slow component, I_{Ks}, encoded by *KCNQ1/KCNE1*, the hook-shaped tail current from the hERG channel established this channel as the most critical myocardial repolarization reserve (Roden and Yang, 2005; Schmitt et al., 2014; Skinner et al., 2019). The full-length protein of the *KCNH2* gene contains 1,159 amino acids (Ono et al., 2020), and mutations at different positions that affect the voltage gating kinetics of the channel are classified as class III mutations (Delisle et al., 2004; Smith et al., 2016; Mehta et al., 2018; Schwartz et al., 2019). Unlike the class II mutations, which mainly lead to intracellular protein maturation defects, class III mutations in different domains of the hERG channel change its gating kinetics mainly manifesting as slowed channel activation or accelerated deactivation. Furthermore, it is well known that the hERG channel is the most common target for arrhythmogenic drugs, which can directly change its gating kinetics or affect the biogenesis of the channel protein. Dysfunction of

the hERG channel caused by congenital gene mutations or drug blockade prolong the duration of the cardiac action potential, which may trigger fatal arrhythmia, such as torsades de pointes (TdP) (Sala et al., 2016; Smith et al., 2016; Mehta et al., 2018; Perry et al., 2020).

Due to the important role of the hERG channel in the formation of cardiac action potentials, exploring the key factors that determine the gating kinetics of the channel and the protein domains involved in gating regulation is expected to suggest therapeutic strategies to address hERG channel dysfunction and its negative gating changes, thus preventing potential severe arrhythmia. However, hERG channel itself is a complicated tetramer structure composed of four subunits with other auxiliary subunits, and site-directed mutagenesis studies have shown that almost every domain located in the full-length channel protein is involved in its gating regulation. These factors affect the development of hERG channel gating regulation drugs. Because of the complicated interactions between the different channel domains, identifying the normal voltage gating mechanism of the hERG channel during myocardial repolarization requires an improved understanding of the role of the involved domains in a conformational change of the channel. Therefore, the high-resolution channel structure on the cell membrane, which shows the location of key regulatory domains, may contribute to the analysis of the gating characteristics of the hERG channel and help us to understand its importance in the action potential.

The Shaker channel, another voltage-gated K^+ (VGK) channel, has provided a basic understanding of the structure and dynamics of the classic cardiac VGK channels (Barros et al., 2019). In addition, site-directed mutagenesis studies have identified important structural domains that regulate the conformational changes of the hERG channel and high-affinity sites for drug binding (Mitcheson et al., 2000; Chen et al., 2002; Perry et al., 2004; Vandenberg et al., 2017). The structure of hERG recently obtained from cryo-electron microscopy, named $hERG_T$, showed subtle structural differences. It provides new insights into the gating mechanism and reveals the molecular basis of drug hypersensitivity (Wang and MacKinnon, 2017) (**Table 1**). Developments in X-ray crystallography and cryo-electron microscopy have revealed the structures of these proteins look in atomic detail but do not tell us how they function. Molecular dynamics (MD) simulation, a powerful forecasting tools, has yielded mechanistic details of fundamental processes that are not available through experiments alone (DeMarco et al., 2019). It has progressed to the point that we can now simulate realistic molecular assemblies to produce quantitative calculations of the thermodynamic and kinetic quantities that control function, such as ion conduction and ion channel gating (Flood et al., 2019). Since the various domains of the hERG channel may be involved in the regulation of its conformational and functional changes, whether induced by congenital gene mutations or drugs, only by combining structural and functional studies of site-directed mutations with MD simulations, etc. can we better understand the underlying complex kinetics (Schewe et al., 2019; Dickson et al., 2020).

For new insights into the structure and kinetics, it is hoped that identifying the key residues that determine the different conformations of the channel and achieving a new understanding of structural differences in $hERG_T$ will enable the correction of hERG channel dysfunction. More importantly, by further identifying which components affect channel gating and how, based on the ability to see the atomic structure inside the ion channel, reversing the dysfunction may require only targeting a single residue located in the channel. Therefore, understanding the molecular level of ion channel physiology has great biomedical significance.

THE MOST IMPORTANT REPOLARIZATION RESERVE

The cardiac hERG channel, also known as the Kv11.1 channel or ERG1 potassium channel, is one of the ether-a-go-go (EAG) potassium channel family members (Barros et al., 2020; He et al., 2020). The proposed sequence of the biosynthesis of hERG channel protein is translation, insertion into the endoplasmic reticulum membrane, the addition of asparagine-linked (N-linked) glycans, the tetramerization of α-subunits, and the correct or native folding of the voltage sensor, pore, NH2 terminus, and COOH terminus (Ono et al., 2020). Mature channel proteins in the membrane become the most critical myocardial repolarization reserve depending on the characteristics of the inward delayed rectifier, which facilitates the completion of depolarization and ensures a normal action potential duration (Roden and Yang, 2005; Skinner et al., 2019).

In addition to congenital mutations that lead to functional loss of the channel, the off-target effects of many drugs target hERG channel to cause current reduction, and the resulting cardiotoxicity is sometimes fatal due to the specific role of the hERG channel in the heart (van Noord et al., 2010). Dysfunctional mutation of the hERG gene causes haplotype deficiency or dominant-negative effects in its protein expression, which can partially or completely reduce the I_{Kr} current and thus prolong the repolarization time course (Egashira et al., 2012; Zhang et al., 2014; Cubeddu, 2016; Ono et al., 2020). Similarly, cardiotoxic drugs bind directly to highly sensitive aromatic amino acid residues (Y652, F656, etc.) in the channel that have a direct blocking effect or prevent the mature expression of channel proteins on the membrane (Vandenberg et al., 2017; Helliwell et al., 2018; Zequn et al., 2021). For example, the antimalarial drugs quinidine and diastereoisomers of quinine show stereoselectivity for the hERG channel, with quinine 14-fold less potent than quinidine, and the hERG-F656C mutation reverses this stereoselectivity, suggesting that residue F656 contributes to the stereoselective pocket for quinidine and quinine (Yan et al., 2016).

The effects of both gene mutations and toxic drugs targeting the hERG channel can be expressed as the QT interval of the electrocardiogram (ECG), and prolongation of the duration beyond the normal range (440 ms for men and 460 ms for women) is clinically called long QT syndrome (LQTS) (van Noord et al., 2010; Schwartz and Ackerman, 2013) (**Figure 1**).

TABLE 1 | Summary of the main structure acquisition and molecular dynamics simulation results mentioned in the paper.

Aim	Methods	Softs or programs	Parameters	Results	Limitation	References
To characterize the high-resolution hERG structure	Cryo-electron microscopy (Cryo-EM)	***Modeling*** Blocres, Bsoft RELION, Frealign ***Generation of atomic model*** HOLE, COOT	***Channel*** hERG channel ($hERG_T$ and $hERG_{Ts}$) ***Force field*** AMBER	The cryo-EM structure of hERG channel-$hERG_T$ of 3.8 Å and its non-inactivating mutant $hERG_{Ts}$ S631A	Low recognition of intracellular structural domains and unable to clarify the mechanism of non-domain exchange	Wang and MacKinnon (2017)
To characterize the structural properties of the cNBD[a] in KCNH2	SEC-MALS[b] SAXS[c] Electrophysiologica-l recording	***Modeling*** PHASER, COOT ***SEC-MALSS*** Astra ***SAXS*** PRIMUS, CRYSOL	***Channel*** hERG channel ***Force field*** -	cNBD of 1.5 Å and a novel salt-bridge E807-R863	No simulation of the function of the salt bridge in different VGK channel[d]	Ben-Bassat et al. (2020)
To explore the VSD/PD[e] coupling mechanism in VGK channels	GIA[f] Network analysis of MD[g]	***Image building*** VMD[h] ***MD*** Carma, Python	***Channel*** Kv1.2/2.1 chimera ***Force field*** -	S4–S5 linker regulates the position of the S6 gates in the Kv1.2/2.1	Unable to accurately estimate the charge of each channel	Fernández-Mariño et al. (2018)
To identify the domains involving the passage of ions through VGK channels	MD Electrophysiologica-l recording	***MD*** CHARMMc36 ***Electro-physiological*** Clampfit 10	***Channel*** KcsA channel ***Force field*** CHARMM	Ligand-induced conformational changes in the KcsA channel removes steric restraints at the SF[i]	No comparison of differences in different penetration mechanisms under different force fields	Heer et al. (2017)
To identify activators that open different potassium channels	X-ray crystallography Cysteine-scanning mutagenesis Atomistic MD	***Crystallography*** **XDS** XSCALE/AIMLESS ***Atomistic MD*** Glide, GROMACS 5.0 and 5.1	***Channel*** K2P channels, VGK BK_{Ca} channels[j] ***Force field*** generalized amber force field (GAFF)	A class of negatively charged activators (NCAs) act as master keys to open K^+ channels gated at their SF	The differences between various potassium channels may challenge this channel opening mechanism	Schewe et al. (2019)
To clarify the mechanism of the passage of ions through VGK channels	X-ray crystallography Extensive molecular MD	***Crystallography*** HKL 2000, PHASER COOT, PHENIX ***MD*** GROMACS 5.1	***Channel*** MthK channel ***Force field*** CHARMM36 AMBER	Channel conductance is controlled at the SF	The simulations go beyond the openings of channel observed in the experiment	Kopec et al. (2019)
To quantitatively elucidate the thermodynamic basis for the AG/SF coupling	Adiabatic energy maps FEP/MD[k]	***Atomic model Construction and MD*** CHARMM	***Channel*** KcsA channel ***Force field*** CHARMM	Phe103 as the critical residue controlling the allosteric AG/SF coupling	FEP/MD only analyzed a single site-directed mutation in detail	Pan et al. (2011)
To understand molecular origins of gating shifts	Solid-state- NMR[l] MD	***NMR*** PISSARROSPARTA ***MD*** NAMD 2.11, Anton	***Channel*** KcsA channel ***Force field*** CHARMM	E71X mutations change the equilibrium between intrinsically sampled filter states	No further experiments to comprehend C-type inactivation	Jekhmane et al. (2019)
To characterize a structural basis for the inactivation mechanism	Homology modeling MD	***Modeling*** Modeller 7v7, pymol ***MD*** Gromacs 3.2	***Channel*** hERG channel ***Force field*** GROMOS96 43a1	Phe627 play a significant role in the hERG C-type inactivation	Shorter MD simulation time cannot replicate the inactivation process of the hERG channel	Stansfeld et al. (2008)
To understand the molecular mechanism of C-type inactivation	MD Electrophysiologica-l recording	***MD*** VMD, NAMD 2.12 NAMD 2.13, Anton ***Electrophysiolog-y*** GPatch	***Channel*** hERG channel ***Ensemble*** NPT ***Force field*** CHARMM	The constricted conformation in hERG involves the residues Y616, N629 and F627	MD still cannot completely solve the unknown molecular mechanism of complex channel regulation	Li et al. (2021)
To simulate specific interactions between VSD and PD	Homology modeling MD	***Modeling*** ClustalW2, TMPred ***MD*** ROMACS 4.5.3	***Channel*** hERG channel ***Force field*** OPLS all-atom	L532 as a structural basis for hERG channel deactivation	The simulation times in fully atomistic models cannot to explore large-scale movement involving	Colenso et al. (2013)

(Continued on following page)

TABLE 1 | (*Continued*) Summary of the main structure acquisition and molecular dynamics simulation results mentioned in the paper.

Aim	Methods	Softs or programs	Parameters	Results	Limitation	References
To understand the mechanism of voltage gating in VGK channel	All-atom MD	***MD*** Anton, HiMach	***Channel*** Kv1.2/2.1 chimera ***Ensemble*** NPT ***Force field*** CHARMM27	To show how a VGK channel switches between activated and deactivated states	The relatively slow calculation speed leads to the system disequilibrium	Jensen et al. (2012)

[a]*cNBD: C-terminal cyclic nucleotide binding domain*
[b]*SEC-MALS: size-exclusion chromatography combined with multi-angle light scattering*
[c]*SAXS: small-angle x-ray scattering*
[d]*VGK channels: voltage-gated K+ channels*
[e]*VSD: voltage sensing domain, PD: pore domain*
[f]*GIA: Generalized Interaction Energy Analysis*
[g]*MD: molecular dynamics*
[h]*VMD: Visual Molecular Dynamics*
[i]*SF: selectivity filter*
[j]*BKCa channels: Ca2+-activated BK-type channels*
[k]*FEP/MD: free energy perturbation/molecular dynamics*
[l]*NMR: Nuclear Magnetic Resonance Spectroscopy, "-" represents "not given"*

Because of the consequent reduced repolarization reserve, excessive prolongation of the QT interval may trigger early after depolarization (EAD) and even induce malignant arrhythmias, such as TdP and ventricular fibrillation (Schwartz & Woosley, 2016).

STRUCTURAL DIFFERENCES FROM HERG$_T$

The hERG channel shares structural homology with other VGK channels (Whicher and MacKinnon, 2016; Vandenberg et al., 2012; Dehghani-Samani et al., 2019). Within contrast to Na^+ channels, the four hERG subunits are cyclically rather than linearly arranged on the membrane to form a tetramer (Vandenberg et al., 2012; Brewer et al., 2020). The hERG channel tetramer is assembled in the endoplasmic reticulum with the help of the human J-protein chaperones DNAJB12 and DNAJB14 (Li et al., 2017). On the membrane, each subunit contains six transmembrane fragments (S1-S6), an N-terminal Per-Arnt-Sim (PAS) domain, and a C-terminal cyclic nucleotide binding domain (cNBD) (Barros et al., 2020; Vandenberg et al., 2012; Shi et al., 2020). S1-S4, as the voltage-sensing domain (VSD), possesses multiple positively charged residues to sense changes in the transmembrane voltage, and which S4 is the most important (Dehghani-Samani et al., 2019; Barros et al., 2012). S1-S3 contain some negatively charged amino acids, which are considered to form the offset charge of the entire VSD. S5-S6 are important structures that constitute the channel pore domain (PD). They form a tetrameric structure surrounding the central conduction pathway by coupling with the middle pore ring of the four subunits and constitute the ion-permeable PD (Barros et al., 2020; Wang et al., 2011). The connection between S4 and S5, the S4-S5 linker, transmits the voltage change information from the VSD to the PD, bridging or translating from the voltage change to physical action (Dehghani-Samani et al., 2019) (**Figure 2A**). It has been reported that the eag domain, which contains the PAS domain and the PAS-Cap domain at the N-terminus, is essential in stabilizing the assembly and trafficking of the hERG subunits (Ke et al., 2014). In addition, mutations at different positions in the cNBD impair the interaction with the eag domain and accelerate the deactivation of the channel (Kume et al., 2018).

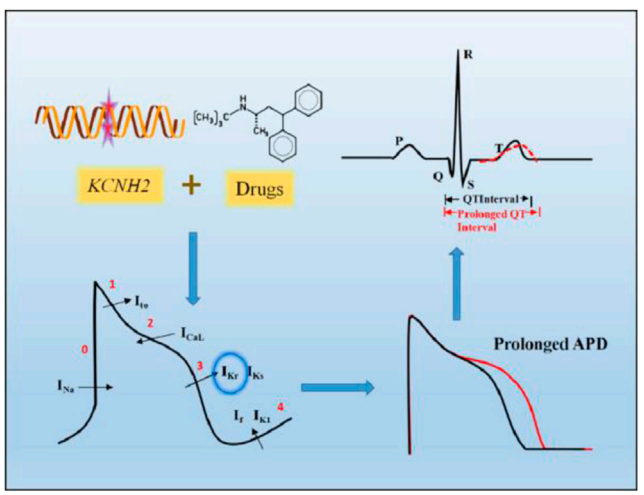

FIGURE 1 | Prolonged QT interval on ECG caused by hERG channel dysfunction. *KCNH2* gene mutations and drugs targeting hERG channel can partially or completely reduce I_{Kr}, which mainly function in the third phase of cardiac action potentials, resulting in prolonged action potential duration (APD), shown on the ECG as a prolonged QT interval.

Recently, the cryo-EM structure of voltage-gated potassium channels has attracted great attention for its great contribution to revealing the gating mechanism and reasons for drug hypersensitivity. The hERG homologous family EAG1 (Kv10.1) structure, which removed 114 residues (773–886)

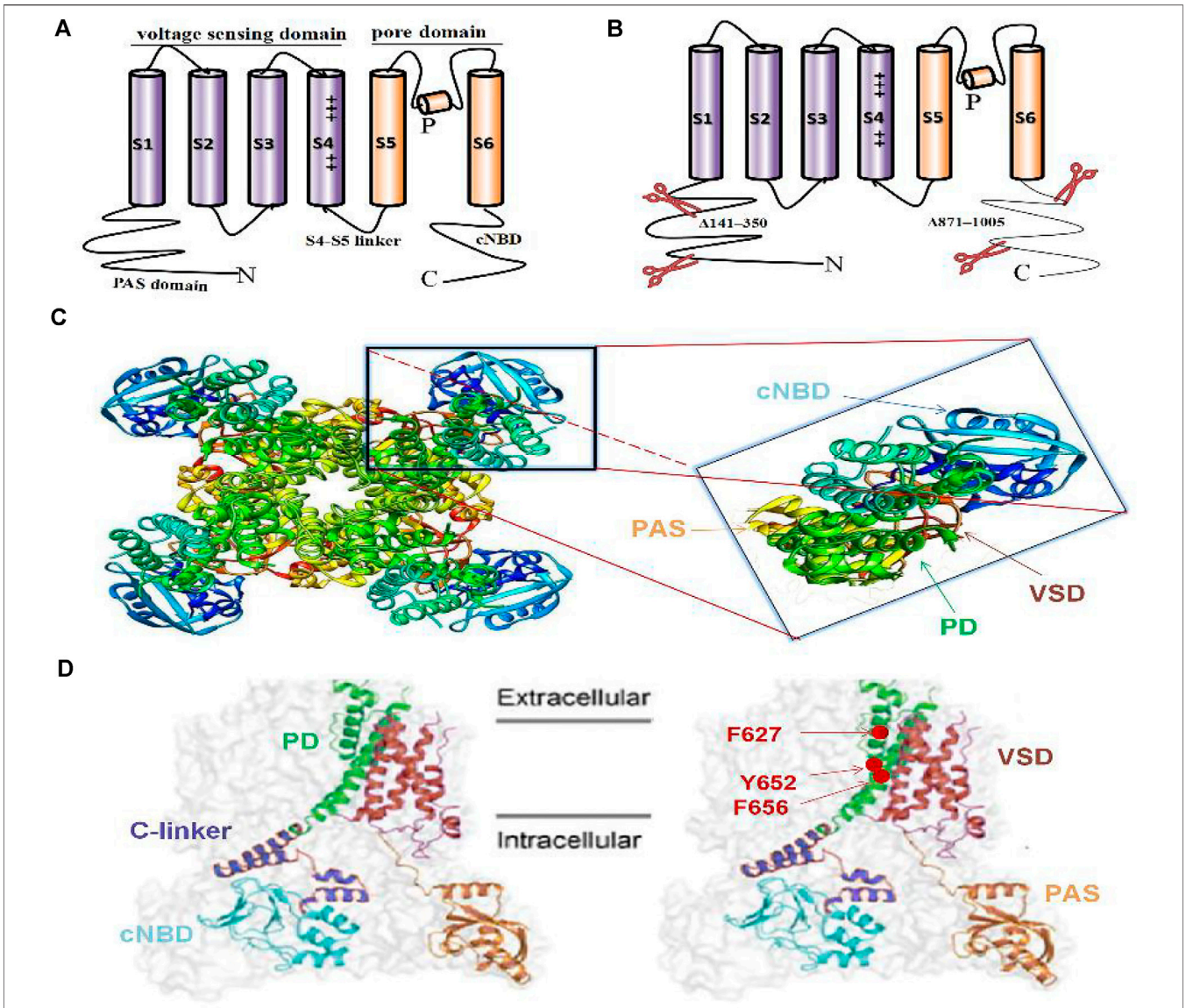

FIGURE 2 | Single alpha subunit of the previous hERG structure **(A)**, new $hERG_T$ structure **(B)**, 3D side view of full $hERG_T$ **(C)**, obtained from PDB: 5VA2; ($hERG_T$) and single subunit of the $hERG_T$ on the cell membrane **(D)**. The classic VGK channel structure contains six transmembrane segments S1-S6, S1-S4 contains charges to form the voltage-sensing domain (VSD), and S5-S6 forms the pore domain (PD) **(A)**. Cryo-electron microscope (cryo-EM) structure $hERG_T$ with deletion of the N-terminal 141–350 and the C-terminal 871–1,005 of the non-structural sequence to prevent aggregation during purification **(B)**. Full-length $hERG_T$ channel contains four subunits arranged in a circular pattern around a central PD (C-left) and magnifying single subunit, colored to distinguish different domains (C-right). The color-marked single subunit corresponding to the above 3D structure on the cell membrane, including the PAS domain at the N-terminal and the C-terminal cyclic nucleotide binding domain (cNBD), transmembrane PD and VSD, as well as the main blocking sites Y652, F656 for specific cardiotoxicity drugs and F627 involving inactivation **(D)**.

from the unstructured C-terminus to achieve a resolution of 3.78 Å, is called rEag1Δ and retains all the characteristics of the full-length channel, including similar gating kinetics (Whicher and MacKinnon, 2016). Subsequently, the hERG cryo-EM structure was obtained: $hERG_T$ was constructed by the deletion of two fragments (N-terminal 141–350 and C-terminal 871–1,005), and the remaining 814 amino acid residues also have similar gating characteristics to the wild-type hERG channel ($hERG_{WT}$) (Wang and MacKinnon, 2017) (**Figures 2B–C–D**). The $hERG_T$ structure has a resolution as high as 3.8 Å and shows subtle differences from the $hERG_{WT}$. First, its S4-S5 linker is the same as the rEag1Δ structure, which is only a short loop containing five residues, not as long as previously thought (Wang and MacKinnon, 2017). Second, unlike the Shaker Kv channel with six or more charged residues, the S4 helix has only five positively charged amino acids, and the first three, K1, R2, and R3, are located outside the cell, while R4 and R5 are inside, indicating that the VSD is in an active state of depolarization (Brewer et al., 2020). In addition, unlike the EAG1 channel, whose pore is closed owing to the presence of Ca^{2+} and calmodulin, the pore in $hERG_T$ is open, and the volume of the central cavity of the channel is very small (Whicher and

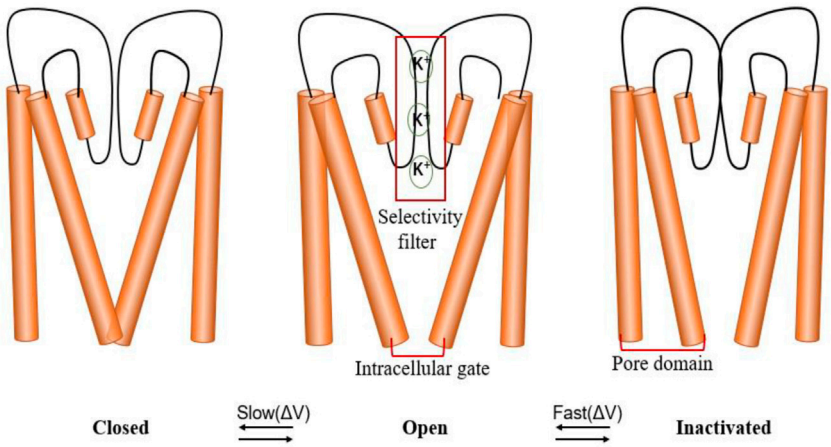

FIGURE 3 | hERG channel gating. The channel has three states: closed, open and inactivated. Schematic diagram of the hERG channel gating scheme, indicating that the state transition between closed and open states is slower than the state transition between open and inactive states. The selectivity filter (SF), located in the central cavity of the pore domain, and the intracellular activation gate (AG) coupling (AG/SF coupling) are thought to participate in different state changes.

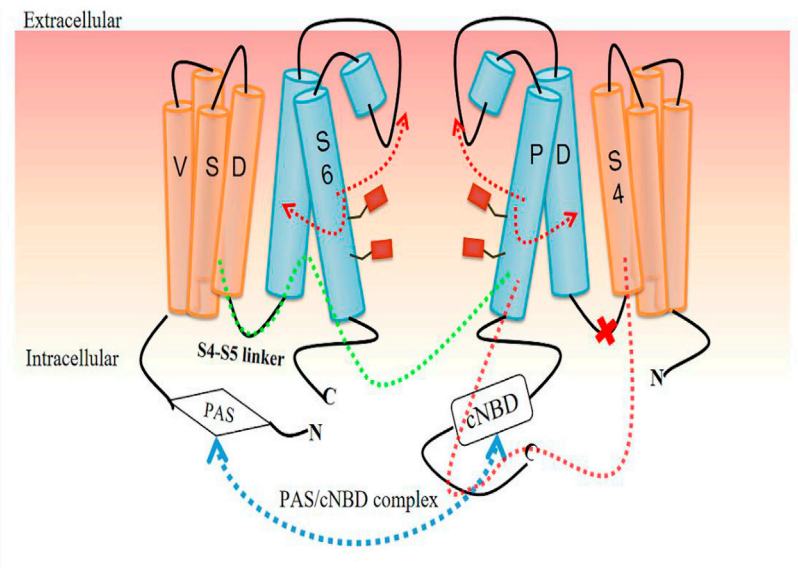

FIGURE 4 | Voltage gating regulation model for hERG channel based on the PAS/cNBD complex. The PAS/cNBD complex plays a central role (*blue dotted line*) in this model. Previously, the S4-S5 linker was thought to act as a bridge to transform the conformational changes in the voltage-sensing domain (VSD) into physical movement of the pore domain (PD) and then interact with adjacent subunits to regulate the pore gate (*green dotted line*). A new insight is that the S4-S5 linker cannot act as a bridge (*red cross*), but S4 of VSD directly interacts with S6 of the same subunit, resulting in a change in the state of S6 followed by opening or closing of the channel gate (*red dotted line and arrow*).

MacKinnon, 2016; Wang and MacKinnon, 2017; Butler et al., 2019).

Notably, there are four deep and elongated hydrophobic pockets stretching into the central cavity to form the selectivity filter (SF), and the drug-sensitive amino acids are located on the surface of these pockets. The well-known hERG channel blockers astemizole and dofetilide embed in the pocket after entering the narrow central cavity. The exposed hydrophobic pocket clearly provides a unique location for drug binding. Furthermore, an extreme negative electrostatic potential is caused by the smaller volume of the central cavity, making it attractive to channel blockers, which contain mainly positive charges, which may be useful to explain the abnormal drug sensitivity of the hERG channel (Wang and MacKinnon, 2017; Butler et al., 2019).

The cryo-EM structure provides groundbreaking insights into the complete channel structure, but its atomic-resolution details

of the intrinsic structural domain of the channel in the cytoplasm are limited. The high-resolution crystal structure of the first hERG channel with a resolution of 1.5 Å identified a functionally vital salt bridge (E807-R863), which did not appear in the cryo-EM structure. Electrophysiological analysis indicated that the salt bridge may not only support the spatial organization of the internal ligands but also maintain the complex interface within the cell (Ben-Bassat et al., 2020) (**Table 1**).

VOLTAGE GATING OF THE HERG CHANNEL

Gating Kinetics in Cardiac Action Potential

Similar to other VGK channels, the hERG channel has three different conformations: closed, open, and inactivated (Vandenberg et al., 2012; Perry et al., 2015; Vandenberg et al., 2017; Zhang et al., 2018; Shi et al., 2020). However, hERG also has unique gating kinetics, that is, rapid inactivation and recovery from inactivation, while activation and deactivation are relatively slow (Vandenberg et al., 2017; Shi et al., 2020). In other words, the inactivation of the hERG channel is more active than the activation, contributing to its inward rectification characteristics (Smith et al., 1996; Spector et al., 1996) (**Figure 3**).

In the early stage of the action potential, the hERG channel opens slowly but inactivates quickly. As the depolarization voltage continues to decrease, the channel gradually recovers from inactivation (Witchel et al., 2001). When the membrane potential is −40 mV, the transmembrane current reaches its peak value. The I_{Kr} current gradually increases during the repolarization process, followed by slow deactivation of the channel (Vandenberg et al., 2012). The channel thus undergoes activation, inactivation, reactivation, and deactivation during the entire course of the action potential, and the channel remains open for a long period (200–300 ms) below the resting potential, resulting in a typical tail current (Barros et al., 2012; Vandenberg et al., 2017).

Key Molecules Involved in the Regulation of Gating Kinetics

The regulation of different conformational changes of hERG channels is so complicated that it is difficult to elucidate the exact mechanism of gating kinetics because almost all domains are involved in this regulation (Codding et al., 2020). Some domains at the C-terminus and N-terminus, particularly the PAS domain and cNBD, have been proven to play key roles in the regulatory mechanism. They may affect the interaction between the PAS domain in one subunit and the cNBD in an adjacent subunit, called the PAS/cNBD complex (Codding et al., 2020; Morais-Cabral and Robertson, 2015; Adaixo et al., 2013) (**Figure 4**). This physical interaction between the different domains of different subunits can produce electrical activity through further coupling, which is a classic explanation for the changes in different conformational states of VGK channels. However, this electromechanical coupling model has been questioned in recent studies (Wang and MacKinnon, 2017; Barros et al., 2019; Butler et al., 2019; Jekhmane et al., 2019; Kopec et al., 2019; Malak et al., 2019).

Homology modeling approaches suppose that protein structure is more conserved than protein sequence. This is usually the case for ion channels, where relatively low 30–40% sequence similarities are accompanied by strong structural resemblance, deviating only by a few angstroms (Forrest et al., 2006). New structural differences from $hERG_T$ and homology models such as KcsA, a prokaryotic channel lacking a VSD that provides an excellent prototypical model system to understand the kinetics of VGK channels, have enabled MD simulations to gradually identify the roles of important gated regulatory molecules.

S4-S5 Linker and Selectivity Filter in Activation

As shown above, the activation process of the hERG channel is very slow, with a voltage threshold of −40 mV to −30 mV, reaching a plateau at +20 mV to +40 mV (He et al., 2020). The physiological significance of slow activation is to minimize the use of channels to reduce antagonism with the inward ion flow of Na^+ and Ca^{2+}(Shi et al., 2020). It is generally believed that the structural rearrangement in VSD is transferred to PD through the physical interaction between the S4-S5 linker and the cytoplasmic end of the S6 helix, opening and closing the activation gate. This conversion of energy from voltage to mechanical motion is called the VSD/PD coupling mechanism (Barros et al., 2020; Shi et al., 2020; Hull et al., 2014; Tristani-Firouzi et al., 2002; Kalstrup and Blunck, 2018) (**Figure 4**). The foundation of this model is that the positively charged residues of S4 move due to the force applied by the transmembrane electric field and become the driving force for the conformational changes in the pore region (Jensen et al., 2012).

Therefore, the existence of the S4-S5 linker as a bridge seems to be necessary for the interaction in different domains to produce conformation change. Additionally, the slow movement of S4 may explain the slow activation. In a recent study combining experiment and MD analysis, a rack-and-pinion type of coupling between VSD and PD involving interactions between helices S4 and S5 was suggested to be a dominant activation process and an alternative mechanism for VGK channels in the Shaker family (Fernández-Mariño et al., 2018, **Table 1**). However, cutting off the S4-S5 linker and thus breaking the connection between VSD and PD did not change the activation kinetics of the channel, indicating that a bridge may not be needed to induce pore opening (Lörinczi et al., 2015; de la Peña et al., 2018) (**Figure 4**). Another piece of supporting evidence arises from the incorporation of fluorescent unnatural amino acid tags at different positions of the S4-S5 linker, which showed that the movement of the linker is not related to the movement of S6, but the channel still opens normally (Kalstrup and Blunck, 2018).

The $hERG_T$ structure also calls the VSD/PD model into question. First, the S4-S5 linker is a short loop containing five residues that cannot act as a mechanical lever to perform domain exchange. Second, the S6 segment lacks the proline-valine-proline (PVP) motif that is considered, in other VGK channels, to narrow the pore region to allow S6 to interact with the S4–S5 linker. Thus, the interaction between VSD and

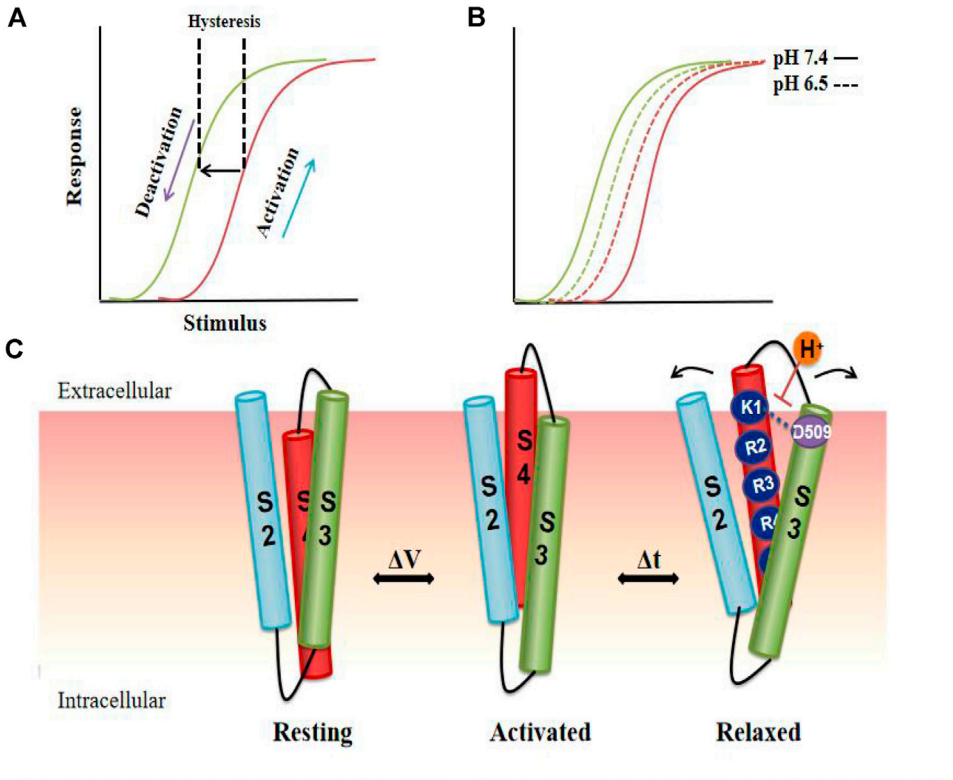

FIGURE 5 | Model of VSD relaxation. In the hERG channel, depolarization activates the channel, and then repolarization causes the channel to deactivate and the VSD to enter a relaxed state, resulting in hysteresis. **(A)** Compared to pH 7.4 (*solid line*), the acidic pH (*dotted line*) shortens the hysteresis behavior of the channel, thereby accelerating the deactivation process. **(B)**. Cartoon representation of proposed reconfigurations of S2, S3, and S4 transmembrane domains in the resting, activated, and relaxed states. S4 in VSD undergoes a conformational change from a stable resting state to a relaxed state due to the voltage change. Putative reconfigurations associated with entering a relaxed state may involve the interaction of D509 with the positive external charge of S4. The neutralization of D509 or external protonation is predicted to destabilize the interactions, thereby destroying the relaxed state. Arrows indicate the hypothetical structural rearrangements of the helix during the relaxation process resulting from the interaction of the acidic residues in S2-S3 **(C)**.

the C-terminus of the S6 segment occurs in the same subunit and does not involve another adjacent subunit (Wang and MacKinnon, 2017). Compared with other VGK channels, the structure of this non-domain exchange shows differences in the movement of the VSD as the mechanism of channel opening (Malak et al., 2017; Malak et al., 2019). Based on this new structural difference, researchers proposed another mode of VSD/PD, showing that S4 directly interacts with the C-terminal joint to cause the bending and relaxation of S6 without the need for the S4-S5 linker (Wang and MacKinnon, 2017). The bending and relaxation of S6 are caused by the different states of S4. When the VSD is in a depolarized state, the S4-S5 linker interacts with the intracellular part of S6 to guide the C-terminus of S4 to the C-terminal joint to relax S6 and open the channel. In contrast, the hyperpolarized state of the VSD causes S6 to bend and thus close the pore gate (Wang and MacKinnon, 2017; Butler et al., 2019) (**Figure 4**).

In summary, the VSD/PD coupling mechanism cannot simply explain the slow activation of the channel. Although the new model provides a possible direction, the exact relationship between the conformational state of VSD and the activation gate remains to be confirmed. Critically, cryo-EM usually has a low resolution for the periphery of the protein structure, which hinders the clear identification of side-chain conformations and the accurate modeling of channel functional characteristics (Herzik et al., 2019). Nonetheless, docking directly to this cryo-EM structure has been reported to yield binding modes that are unable to explain known mutagenesis data. Therefore, further MD simulation may be an effective tool to support the above hypothesis and produce consistent data.

In potassium channels, predominately in the context of C-type inactivation, the allosteric coupling between the activation gate (AG) and the SF has been studied for a long time. This coupling was proposed to play a role in the closed-to-open transition, where upon initial opening, the AG affects the SF by changing its conformation from prime-to-conduct to conductive (Heer et al., 2017) (**Figure 3**). MD simulations and electrophysiology measurements showed that ligand-induced conformational changes in the KcsA channel remove steric restraints at the SF, resulting in structural fluctuations, reduced K^+ affinity, and increased ion permeation. Such activation of the SF may be a universal gating mechanism within VGK channels (Heer et al., 2017, **Table 1**).

More recently, a class of negatively charged activators (NCAs) have been reported to act as master keys to open potassium channels (K2P, BK, and hERG) gated at the SF via a conserved but not specific mechanism. Through functional analysis, X-ray crystallography, and MD simulations, the NCAs were shown to bind to similar sites below the SF, increase pore and SF K^+ occupancy, and open the filter gate. In addition, MD simulations of the calcium-gated prokaryotic potassium channel MthK provide further evidence to support AG/SF coupling. Therefore, several structurally distinct potassium channels were postulated to be activated/gated at the SF (Schewe et al., 2019, **Table 1**). The motions of the pore-lining TM helices constituting the AG underlie the narrowing and widening motions of the SF, predominately at residue T59, the equivalent threonine in KcsA, which in turn controls the ionic flow through the channel (Kopec et al., 2019, **Table 1**). In summary, the channel's conductance is regulated at the SF through allosteric coupling with the AG (Labro et al., 2018). Thus, targeting a specific residue in the SF to design activators of the hERG channel may reverse the steric hindrance effect of drugs that bind directly to the channel.

Selectivity Filter and F627 in Inactivation

After the hERG channel is slowly activated, an abnormally rapid inactivation process occurs, and the channel can be inactivated within 1–2 ms at +60 mV (Shi et al., 2020). Rapid inactivation and recovery from inactivation maintain the steady state of the action potential (Perry et al., 2015). The inactivation of the hERG channel is essentially of P/C type because it is not affected by N-terminal deletion (Schönherr and Heinemann, 1996). This type of inactivation is sensitive to the ion occupancy in the SF, indicating that inactivation may involve the structural rearrangement of the SF (Schönherr and Heinemann, 1996). A selective and rapid flux of K^+ across the cell membrane through a central pore is regulated by the interplay between an AG and a C-type inactivation gate known as SF, called activation coupled to C-type inactivation (Tilegenova et al., 2017; Jekhmane et al., 2019; Kopec et al., 2019).

MD simulations of the pore helix and SF regions of the hERG channel based on KcsA have provided insights into the molecular basis of inactivation (LeMasurier et al., 2001). Using free energy perturbation MD simulations, Phe103, a residue located along the inner helix, was identified as the critical residue controlling the allosteric coupling between SF and AG (Pan et al., 2011, **Table 1**). Furthermore, T75A in KcsA proves that, upon activation, the SF transitions from a nonconductive and deep C-type inactivated conformation to a conductive conformation (Labro et al., 2018).

In VGK channels, the term mode shift or hysteresis determines a normal heartbeat and regulates cell excitability (Männikkö et al., 2005; Corbin-Leftwich et al., 2016). One example of the physiological importance of hysteresis is in the regulation of the activation, deactivation, and inactivation gating of voltage-gated Na^+ channels and VGK channels (Villalba-Galea, 2017). Initially, the time course of development of the mode shift was correlated with that of P/C-type inactivation, suggesting that P/C-type inactivation is required for mode shift to occur (Olcese et al., 1997). Hysteresis in tetrameric cation-selective channels can arise from AG/SF allosteric coupling, and mode shift was prevented in the total absence of C-type inactivation, indicating that SF plays an essential role in activation-inactivation coupling (Tilegenova et al., 2017) (**Figure 3**). By integrating solid-state NMR and MD simulations, the E71 point mutation E71X was shown to rearrange the network behind the SF and perturb the K^+ binding sites V76 and Y78, thus changing the equilibrium between the intrinsically sampled filter states (Jekhmane et al., 2019, **Table 1**). Interestingly, the Y78 conformation can change in reference to the filter mode, which is consistent with a recent cryo-EM structure of the hERG channel (Cheng et al., 2011; Wang and MacKinnon, 2017).

Unlike other VGK channels, the SF part of the hERG channel located on PD is not conserved and has some slight sequence differences. Five residues in KcsA, Trp67, Trp68, Glu71, Tyr78, and Asp80, have been proposed to be involved in hydrogen bond networks that stabilize the structure of the SF in the inactivated states. The equivalent residues in the hERG channel are Tyr616, Phe617, Ser620, Phe627, and Asn629, and none of them is conserved (Vandenberg et al., 2012). Notably, C-type inactivation is governed by a complex hydrogen bond network behind the SF (Cordero-Morales et al., 2006). The variable residue-induced lack of hydrogen bond networks in the channel contributes to the greater inclination of the SF to "collapse", leading to inactivation (Xu and McDermott, 2019). Specific mutations of SF residues may change the inactivation characteristics of hERG channels (Schönherr and Heinemann, 1996; Wang et al., 2011).

In MD simulations, several residues in the SF were displaced in the pore axis, especially F627 in the GFG motif (Stansfeld et al., 2008, **Table 1**). The positional specificity of F627 was confirmed under the cryo-EM structure. Compared to the ERG1 channel, the direction of F627 in $hERG_T$ is offset. A non-inactivated S631A mutant structure $hERG_{Ts}$ S631A to simulate the non-transient inactivation of other Kv channels showed that the position of F627 in the SF is similar to that in the other channels (Whicher and MacKinnon, 2016; Wang and MacKinnon, 2017). The unique position of F627 represents the subtle differences in the SF conformation, suggesting that hERG may be rapidly inactivated by adjusting a single residue. At the same time, the great subtlety of the conformational change of SF is also emphasized. When potassium ions are observed to flow out of the SF, the changes are different from the larger conformational changes of KcsA K^+ channels (Brewer et al., 2020). The latest results from a long-time scale MD support the key role of SF and F627, which demonstrates that the asymmetrical constricted-like conformation of the SF and the side chain rotation of F627 and the hydrogen bond between Y616 and N629 are key determinants for the a C-type inactivation (Li et al., 2021, **Table 1**). More structural and simulation evidence is needed to further support the regulation of hERG channel inactivation through a single residue, which is of utmost significance for research on inhibitors that accelerate channel inactivation.

Voltage-Sensing Domain in Deactivation

Following rapid recovery from inactivation, the hERG channel undergoes slow deactivation. Deactivation represents the closing

of the hERG channel and is an important landmark feature. The regulation of deactivation is so complicated that it cannot be explained by the slow movement of the VSD, because it involves multiple regions of the channel. The interaction between the N-terminal Cap domain (N-Cap), PAS, and cNBD is critical for regulating slow deactivation (Muskett et al., 2011; Ng et al., 2011; Gianulis et al., 2013). The mutation of N-Cap accelerates the deactivation of the channel (Muskett et al., 2011). The application of a PAS domain fragment (1–135) restores slow deactivation to N-terminally deleted (Δ2–354) fast-deactivating hERG channels (Gustina and Trudeau, 2012; Gustina and Trudeau, 2013). And mutations at different positions in the cNBD impair the interaction with the eag domain and accelerate the deactivation of the channel (Kume et al., 2018). These results indicate that the PAS/cNBD complex occupies a central position in the regulation of deactivation, and the eag domain containing N-Cap and PAS is particularly prominent.

Consistently, the $hERG_T$ structure further proves that the N-terminus is indeed integrated on the VSD/PD interface, indicating that the N-terminal PAS domain may move to the plasma membrane during the slow deactivation process, altering the VSD/PD interaction (Barros et al., 2019). It is worth mentioning that in a study of HEK293 cells expressing wild-type (WT) or $hERG_T$ channels, the deactivation rate was significantly slower for $hERG_T$ cells whose two cytoplasmic regions were deleted, which may reflect the influence of changes to electrostatic interactions on the VSD (Zhang et al., 2020).

The pairwise comparison of wild-type and mutant channel models is considered to be a useful method to explain the uncertain functional data in the model structure. In this approach, a 0.5 μs MD simulation indicated that the hERG-L532P mutation reduced the extent of interaction across the S4-S5 interface, suggesting a structural basis for the greatly enhanced deactivation rate (Colenso et al., 2013, **Table 1**). All-atom MD simulations showed how a VGK switches between activated and deactivated states. Upon deactivation, pore hydrophobic collapse rapidly halts ion flow. Subsequent VSD relaxation, including a 15 Å inward S4 helix motion, completes the transition (Jensen et al., 2012, **Table 1**). Future MD simulations will likely be used to assess the validity of these assumptions.

As mentioned above, hysteresis can be observed in voltage-gated ion channels during each activation and deactivation cycle. The terms hysteresis and mode shift are often used to describe the separation between VGK channel activation and deactivation. Similar hysteresis behavior has been reported in a variety of potassium channels, such as KcSA (Tilegenova et al., 2017), Shaker (Labro et al., 2012; Priest et al., 2013), Kv3.1 (Labro et al., 2015), Kv7.2/7.3 (Corbin-Leftwich et al., 2016), Kv11.1 (hERG) (Goodchild et al., 2015; Shi et al., 2019) and Kv12.1 (Dierich et al., 2018). Relaxation of the VSD was identified as a reason for the slow deactivation of the hERG channel (Shi et al., 2020; Jensen et al., 2012; Shi et al., 2019). It is an inherent property of the VSD to be stabilized in a relaxed conformation after the hERG channel is activated. Relaxation causes energy separation of the channel activation and deactivation pathways, resulting in hysteresis and thus regulating the deactivation gating (Shi et al., 2019) (**Figure 5**). A dynamic model describing hERG channel VSD relaxation also shows that the instability of the VSD relaxation state, caused by extracellular protons, may drive voltage sensor return, leading to acceleration of the deactivation process (Shi et al., 2019; Wilson et al., 2019). In this model, acidic pH reduces the mode-shift behavior in hERG channels by destabilizing the relaxed state of the VSD, which is mediated by an extracellular acidic site, D509 (**Figure 5**). As an important contributor to slow deactivation, VSD relaxation could perhaps act as a master switch influenced by mutations within the N-terminus that stabilize the activated voltage sensor in a relaxed state (Goodchild et al., 2015; Thouta et al., 2017). Importantly, mode shift in the hERG channel occurs on a physiological time scale, suggesting that the voltage-dependent dynamic switching of activation and deactivation gating may contribute to the amplitude and timing of the repolarizing I_{Kr} current during the cardiac action potential.

Regulating the deactivation gating kinetics of hERG channels seems to be particularly important. Drugs that act as channel blockers or activators to alter this process by acting on the corresponding domain would produce an apparent pathogenic or therapeutic effect (Sala et al., 2016; Perry et al., 2020). Furthermore, developing more effective channel activators requires more detailed information about the role of the eag domain, VSD, and PD.

SUMMARY AND PERSPECTIVES

There are a variety of potassium channels involved in regulating the action potential of cardiomyocytes, mainly including voltage-gated channels and ligand-gated channels. As a member of the VGK channels, the hERG channel has attracted much attention for its unique gating kinetics and its high affinity for cardiotoxic drugs. Early site-directed mutagenesis studies helped us identify which key amino acid in the hERG channel determines the above characteristics. New progress based on structural and MD simulation models, showing subtle differences from previous classic structures, has even allowed us to understand how the hERG channel functions and why it shows a higher affinity to some specific drugs.

Compared with drugs that affect the maturation of hERG channel proteins to decrease the I_{Kr} current, drugs that bind directly to the amino acid sites of the hERG channel to change the gating kinetics result in a more rapid channel failure. As mentioned above, these direct effects may sometimes produce extremely serious or even fatal cellular or clinical phenotypes (Zequn et al., 2021).

Currently, although novel structural views and some MD simulations with high numerical accuracy, they still cannot solve all the known and unknown factors that affect the actual hERG kinetics determined by the inherent complexity of biofilm channels (Li et al., 2021). These are limited by the availability of the structure and the limitations of MD simulation. Unlike the costly data obtained from experiments, computer simulations with lower cost will allow for quantitative hypothesis testing of how altered hERG functions. However, under different conditions of simulations, such as time, temperature, force

field, and algorithm selection of MD, the opposite results may generate. Therefore, only by combining the high-resolution structure, more accurate MD, and experimental results, detailed studies of the hERG channel gating enable the generation of biophysically accurate models of the hERG gating kinetics. Incorporation of these molecular-level models into cells and ultimately whole hearts that will permit more informed predictions of how the hERG channel functions under physiological or pathological conditions (Perry et al., 2015). And it will bring us a molecular perspective of channel gating state changes, and by targeting these key molecules it can produce corrective effects on the hERG channel, such as activation (Garg et al., 2013; Zangerl-Plessl et al., 2020), enhancement (Wu et al., 2014; Donovan et al., 2018), and allosteric regulation (Sala et al., 2016; van Veldhoven et al., 2021).

AUTHOR CONTRIBUTIONS

ZZ: Conceptualization; Data interpretation; Writing- Original draft preparation; Software; Resources. LJ: Conceptualization; Writing-review & editing.

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