Enhancement of hERG channel activity by scFv antibody fragments targeted to the PAS domain

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The human human ether-à-go-go–related gene (hERG) potassium channel plays a critical role in the repolarization of the cardiac action potential. Changes in hERG channel function underlie long QT syndrome (LQTS) and are associated with cardiac arrhythmias and sudden death. A striking feature of this channel and KCNH channels in general is the presence of an N-terminal Per-Arnt-Sim (PAS) domain. In other proteins, PAS domains bind ligands and modulate effector domains. However, the PAS domains of KCNH channels are orphan receptors. We have uncovered a family of positive modulators of hERG that specifically bind to the PAS domain. We generated two single-chain variable fragments (scFvs) that recognize different epitopes on the PAS domain. Both antibodies increase the rate of deactivation but have different effects on channel activation and inactivation. Importantly, we show that both antibodies, on binding to the PAS domain, increase the total amount of current that permeates the channel during a ventricular action potential and significantly reduce the action potential duration recorded in human cardiomyocytes. Overall, these molecules constitute a previously unidentified class of positive modulators and establish that allosteric modulation of hERG channel function through ligand binding to the PAS domain can be attained.

potassium channel | hERG | KCNH | PAS domain | scFv

The human ether-à-go-go–related gene (*hERG*; or *KCNH2*) encodes the voltage-gated potassium channel that conducts I_{Kr} (delayed rectifier potassium current), a critical cardiac repolarizing current (1, 2). Mutations in *hERG* (3) or channel block can cause long QT syndrome (LQTS) and catastrophic ventricular arrhythmias (reviewed in ref. 4). Because most drugs causing acquired LQTS block I_{Kr} channels (5), lead compounds in development are counterscreened using a hERG cell-based safety test (6) to reduce the incidence of sudden cardiac death caused by off-target drug effects (7).

A defining feature of hERG and its relatives in the potassium channel family, KCNH (8), is a large cytoplasmic region with an N-terminal Per-Arnt-Sim (PAS) domain (9). Within the hERG PAS domain, there are two functionally and structurally distinct regions: the PAS-Cap (residues 1–25) and the globular region (residues 26–135). NMR and crystallographic structures of the isolated PAS domain show that the PAS-Cap region is partially unstructured (10–12). The globular region interacts with a C-terminal domain, which, because of sequence homology with cyclic nucleotide binding domains, is termed the cyclic nucleotide binding homology (CNBh) domain (13, 14). Truncations and mutations of the PAS-Cap and PAS clearly affect the gating mechanisms of the channel, which is particularly apparent in the deactivation kinetics (9, 15–18). PAS domains within the tetrameric hERG channels exert a suppressive effect by slowing channel activation and recovery from inactivation, the two parameters that predominantly determine current amplitude during an action potential (AP) (19). These properties are genetically tuned in native I_{Kr} produced by the heteromeric association of hERG1a (an isoform containing the PAS domain) with hERG1b (an isoform lacking the PAS domain) (19–22). In addition, evolutionary tuning of the properties of these channels by the presence or absence of the PAS domain has been described (23). The functional importance of the PAS domain is reinforced by genetic alterations in the domain that have been linked to LQTS (15, 24, 25).

Because of the physiological role of hERG and its link to LQTS, there has been great interest in understanding the modulatory mechanisms of the channel and the functional role played by its cytosolic domains. From this perspective, the PAS domain is particularly interesting because, in other proteins, these domains bind small molecules and modulate protein activity (26). However, no such ligands have been identified for the PAS domain in hERG or for any member of the KCNH superfamily. In fact, we have recently reported that two different small molecule screening campaigns failed to yield PAS domain-specific binders (27). Importantly, despite extensive work showing that mutations and truncations of hERG PAS cause functional changes, it remains unclear if this domain mediates allosteric regulation in the channel (28).

In search of novel tools to interrogate the functional role of the hERG PAS domain, we generated single-chain variable fragment (scFv) antibodies that recognize this domain and found that these protein molecules change the functional properties of both heterologously expressed and native hERG channels. scFv antibodies consist only of the variable light (V_L) and variable heavy (V_H) antibody domains and therefore are relatively small (27 kDa) but retain their high antibody specificity. Two of the scFv molecules exhibit differential effects reflecting their interaction with different regions of the PAS domain, allowing us

Significance

The human ether-à-go-go–related gene (hERG) potassium channel has an important role in controlling heartbeat. Genetic alterations or drug side effects can reduce the amount of current passing through hERG, prolonging action potential duration and causing irregular heartbeat and sudden death. We uncovered a family of hERG modulators, antibody fragments that bind to a cytoplasmic region [Per-Arnt-Sim (PAS) domain], increasing the magnitude of the repolarizing current I_{Kr} and reducing duration of the ventricular action potential. Using the antibodies as tools, we established that it is possible to modulate hERG channel function through the PAS domain and showed a way to increase hERG channel function with the potential to treat not only perturbations of the channel itself but also arrhythmias arising from heart failure.

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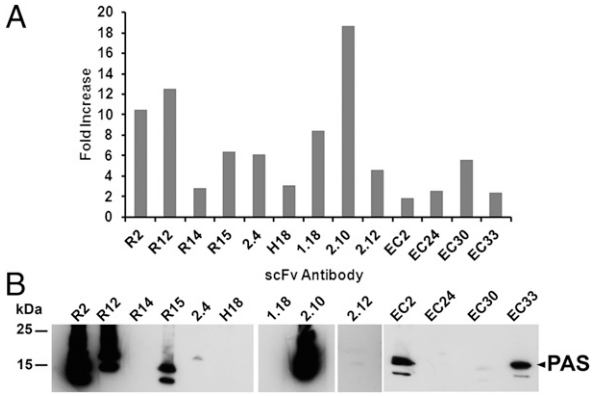

Fig. 1. Characterization of clones by ELISA and Western blot overlay. (*A*) ELISA response for scFv binding to PAS protein over GST control protein expressed as fold increase in OD at 450 nm. All samples were run in duplicate. (*B*) Western blot showing scFv lysate recognition of unfolded PAS. Blot was overexposed to determine if scFv clones recognize blotted PAS protein.

to explore the properties of the PAS domain within the context of the full-length channel.

Results

Creation of scFv Molecules Against the PAS Domain. To obtain antibody molecules against the PAS domain from hERG, we first immunized chickens with pure protein (hERG residues 1–135) expressed in *Escherichia coli*. Total mRNA was isolated from bone marrow and spleen to generate cDNA, and the V_L and V_H regions of IgY were amplified. An scFv phage-display library was generated where the V_L IgY regions were fused to the V_H IgY regions through a linker, forming a single polypeptide, which could be displayed on the surface of phage particles (details are in *SI Materials and Methods*) (29, 30). These scFv molecules were screened for their ability to bind PAS domain using two different strategies. For both strategies, four rounds of selection and amplification were carried out in duplicate or triplicate, yielding between 2,000- and 3,000-fold enrichment in the number of eluted phage (Table S1). In total, 136 individual colonies were randomly picked from the fourth round of screening from both strategies and subjected to DNA sequencing. In parallel, DNA was transformed into the nonsuppressor *E. coli* strain BL21(DE3) for the expression of scFv proteins in the periplasmic space. Crude lysates were generated and used in an ELISA to measure binding specificity to PAS protein over a GST protein control or a Western blot overlay assay to assess their ability to recognize denatured PAS protein. As shown in Fig. 1*A*, a variety of responses was seen in ELISA, and positive hits were defined as showing a greater than or equal to fivefold increase in binding to PAS protein compared with GST alone. Interestingly, Western blot overlay experiments of the same samples revealed that one-half of the scFv antibodies (R2, R12, R15, 2.10, EC2, and EC33) recognize denatured PAS protein, whereas one-half did not (R14, 2.4, H18, 2.12, EC24, and EC30) (Fig. 1*B*). Comparison of the results from the Western blot overlay assay (unfolded PAS protein) and ELISA (folded PAS protein) shows that some scFv molecules only recognize epitopes in the folded state of the protein. For example, we did not detect a Western blot signal for scFv 2.12; however, in ELISA, scFv 2.12 gives rise to a clear signal.

Functional scFv Screen. A group of three scFv molecules (R15, 2.10, and 2.12) was expressed in *E. coli* and purified, and their functional impact on the hERG channel was evaluated at room temperature (22 °C) in HEK293 cells expressing hERG 1a (Fig. 2 and Table S2). Control currents measured using whole-cell patch clamp were compared with those in which the individual antibodies were perfused via the patch pipette into the cytosol in separate experiments. The majority of the scFvs accelerated

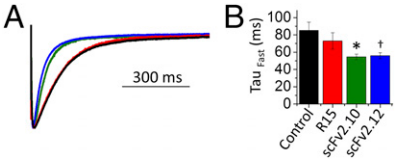

Fig. 2. scFv antibodies accelerate deactivation. (*A*) Whole-cell patch clamp recordings at 22 °C in HEK293 cells expressing hERG 1a. Antibodies were delivered to the cytoplasm via the pipette. (*B*) Fast time constants of deactivation at −110 mV for control (black), scFv 2.10 (green), scFv 2.12 (blue), and scFv R15 (red). Mean ± SEM (n = 11–17). *Statistical significance between vehicle control and scFv 2.10 at $P < 0.05$. †Statistical significance between vehicle control and scFv 2.12 at $P < 0.05$.

channel deactivation as if they perturb the PAS domain that maintains slow deactivation (9). The more modest effect of the antibodies compared with PAS deletions suggests the antibodies do not fully disrupt PAS function. Importantly, one of the selected scFv molecules (R15) did not alter the deactivation properties, thus serving as a negative control. As expected, an antibody directed against the unique N terminus of hERG 1b (22) did not affect hERG 1a deactivation (Fig. S1). Fitting a double exponential function to the deactivation time course evoked at −110 mV showed that the fast time constant of current decay (τ_{fast}) was significantly reduced (Fig. 2*B*, Fig. S1, and Table S2). The slow time constant of current decay (τ_{slow}) was not significantly affected (Fig. S1 and Table S2).

Binding Properties of scFv 2.10 and scFv 2.12 Antibodies. We used biochemical approaches to better understand the modes of action of the scFv 2.10 and scFv 2.12 antibodies on the hERG channel. We determined their binding affinities for the PAS protein using isothermal titration calorimetry (ITC). Titrations of PAS domain into scFv 2.10 antibody revealed an exothermic association with a K_d of 254 nM (Fig. 3*A* and Table 1). The

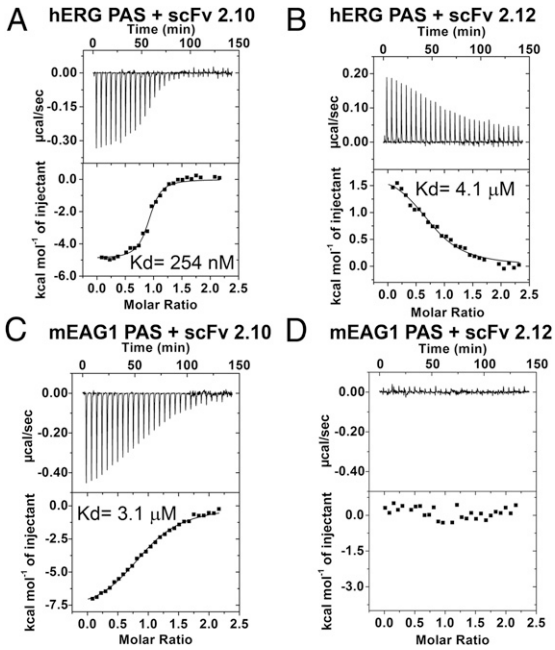

Fig. 3. ITC measurements of antibody and PAS domain. Thermograms recorded at 15 °C. (*A*) hERG PAS protein titrated into scFv 2.10_{HisHA}. (*B*) hERG PAS protein titrated into scFv 2.12_{HisHA}. (*C*) mEAG1 PAS protein titrated into scFv 2.10_{HisHA}. (*D*) mEAG1 PAS protein titrated into scFv 2.12_{HisHA}. Averaged K_d values are shown. Panels show injections of 10 µL titrant to the target (*Upper*) and binding isotherms (*Lower*). Titrations were repeated at least twice.

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Table 1. ITC parameters for scFv binding to hERG PAS and mEAG1 PAS

	hERG PAS		mEAG1 PAS
	scFv 2.10	scFv 2.12	scFv 2.10
K_d (μM)	0.254 ± 0.09	3.92 ± 1.11; 4.31 ± 0.81	3.11 ± 0.02
ΔH (kcal/mol)	−5.06 ± 0.13	1.67 ± 0.12; 1.82 ± 0.11	−8.98 ± 1.172
−TΔS (kcal/mol)	−11.0 ± 0.2	8.79; 8.90	1.72 ± 1.10
N	0.92 ± 0.03	0.93 ± 0.05; 0.81 ± 0.03	0.84 ± 0.18
n	3	2	3

Means ± SD are shown, except for scFv 2.12, where values (and fit errors) of individual measurements are shown. *N* is stoichiometry of binding, and *n* is number of replicates.

association of PAS protein with the scFv 2.12 antibody is endothermic and shows a lower affinity, with a K_d of 4.1 μM (Fig. 3*B* and Table 1). In both cases, stable complexes formed with 1:1 binding stoichiometry as derived from the midpoint of the ITC titrations. The differences in the thermodynamic parameters give an indication of different modes of binding by the two antibodies to the PAS domain.

We also determined how specific these antibodies were for the hERG channel. For this purpose, we used the PAS domain of the mouse ether-à-go-go 1 (mEAG1) channel. The mEAG1 PAS domain is ∼38% identical to the PAS domain of the hERG channel and ∼98% identical to the domain in the human EAG1 channel. The binding of scFv 2.10 to the mEAG1 PAS domain was exothermic, which was previously seen for hERG PAS, but the affinity of binding was 10-fold lower, with a K_d ∼ 3 μM (Fig. 3*C* and Table 1). This effect resulted from a change in binding entropy, which is positive (−TΔS) in the interaction with the hERG domain and negative in the interaction with the mEAG1 domain. No interaction was detected for the titration of mEAG1 PAS with the scFv 2.12 molecule, despite performing titrations at various temperatures (Fig. 3*D*).

To define the binding epitopes on the PAS domain for the two antibodies, we performed in vitro pulldown experiments using different N-terminal truncations (Fig. 4 *A*, *Upper* and *B*, *Upper*). Glutathione magnetic beads were saturated with purified N-terminal GST fusions of (*i*) full-length PAS (N 1–135), (*ii*) deletion of the first 9 residues of PAS (N 10–135), and (*iii*) deletion of the first 23 residues of PAS (N 24–135). With these truncations, we removed either the first section of the PAS-Cap, which is disordered in the NMR structure, or the entire PAS-Cap region. Beads saturated with GST alone were used as a control. We incubated the beads with equal amounts of purified His-tagged scFv 2.10 (Fig. 4*A*) or scFv 2.12 protein (Fig. 4*B*) and after thorough washing, determined if antibody was bound to the beads. The beads were analyzed by SDS/PAGE and Western blot. As shown by Coomassie staining of the gels (Fig. 4 *A*, *Upper* and *B*, *Upper*), all GST fusions were present on the beads in equal amounts. The scFv 2.10 antibody bound only when incubated with the full-length PAS protein; even a small deletion of the first nine amino acids was sufficient to interfere with antibody binding (Fig. 4*A*, *Lower*). In contrast, scFv 2.12 antibody bound to all of the GST fusion proteins (Fig. 4*B*, *Lower*). These results suggest that scFv 2.12 binds to the globular region of the PAS, whereas scFv 2.10 binds to the first nine residues of the PAS-Cap region. We also performed pulldown experiments with the mEAG1 full-length and N-terminally truncated PAS domain and observed similar effects (Fig. S2). To better define the hERG PAS residues involved in the interaction with 2.10, we mutated conserved residues in PAS-Cap, R4, R5, and F14 (Fig. 4*C*). Pulldown experiments show that the double mutants R4E/R5E or R4A/R5A drastically affect the interaction with scFv 2.10, whereas the F14A mutation does not, showing that the conserved residues in the initial section of the PAS-Cap are part of the 2.10 epitope.

Gating Effects of scFv Antibodies on hERG Current. To determine whether the differential targeting of the scFv 2.10 and scFv 2.12 antibodies exerts a corresponding difference in function, we analyzed their effects on hERG currents heterologously expressed in HEK293 cells and recorded at physiological temperature (36 °C ± 1 °C). We applied the antibodies to the cytoplasm via the patch pipette at saturating concentrations of 10 μM and found that scFv 2.10 and scFv 2.12 differentially modulated activation and inactivation kinetics. scFv 2.12 significantly accelerated the time course of activation (Fig. 5 *A* and *B* and Table 2) and inactivation recovery (Fig. 5 *C* and *D* and Table 2) relative to vehicle controls. scFv 2.10 significantly slowed the time course of inactivation onset (Fig. 5 *E* and *F* and Table 2) but had no effect on channel activation or inactivation recovery. Curiously, the antibodies had no effect on deactivation at physiological temperatures (Table S2). Traces showing endogenous currents during key protocols from untransfected HEK293 cells for comparison are provided in Fig. S3. These results define distinct mechanisms by which the scFv 2.10 and scFv 2.12 antibodies modulate hERG channel gating through the PAS domain.

hERG-Interacting scFv Antibodies Enhance hERG Current. We next determined the consequences of the changes in gating induced by scFv 2.10 and scFv 2.12 antibodies on current amplitude both at steady state and in response to a voltage command mimicking the ventricular AP. It is apparent from the examples in Fig. 6*A* that both antibodies increased the magnitude of the steady-state current, which is shown normalized to the peak tail current evoked at −50 mV (Fig. 6 *A* and *B*). The antibodies had little effect on the conductance–voltage plot for activation (Fig. 6*C*, *SI Materials and Methods*, and Table S3), but both shifted the voltage dependence of inactivation by ∼15 mV (Fig. 6*D*, *SI Materials and Methods*, Fig. S4, and Table S3).

To predict how these changes would affect current during a ventricular AP, we measured "repolarizing charge" (*Q*) from currents elicited during a voltage protocol that mimics a human ventricular AP (Fig. 6*E*). Relative repolarizing charge was calculated as the integral of membrane current elicited during the AP protocol normalized to the maximum peak tail current recorded from the same cell after a saturating voltage step (19). Both antibodies significantly increased the total repolarizing charge compared with control (Fig. 6*F*). The differential effect

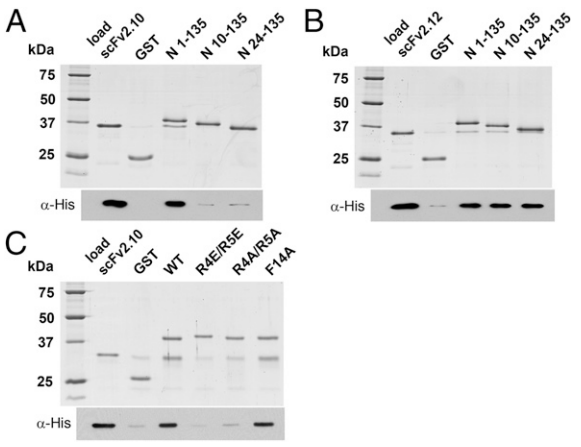

Fig. 4. Epitope mapping of scFv interaction with PAS domain. Pulldowns of (*A* and *C*) scFv 2.10_{HisHA} or (*B*) scFv 2.12_{HisHA} with GST alone or GST fusions attached to beads. Fusions include full-length hERG PAS domain (residues 1–135:N 1–135 or WT), domain truncations (residues 10–135:N 10–135 and 24–135:N 24–135), and mutants of full-length domain (R4E/R5E, R4A/R5A, or F14A). *Upper* depicts Coomassie staining of proteins retained in beads. *Lower* represents Western blots (with an anti-His tag antibody) of scFv retained in beads. Initial load sample of scFv proteins is included. Two independent experiments were performed, each with two replicates.

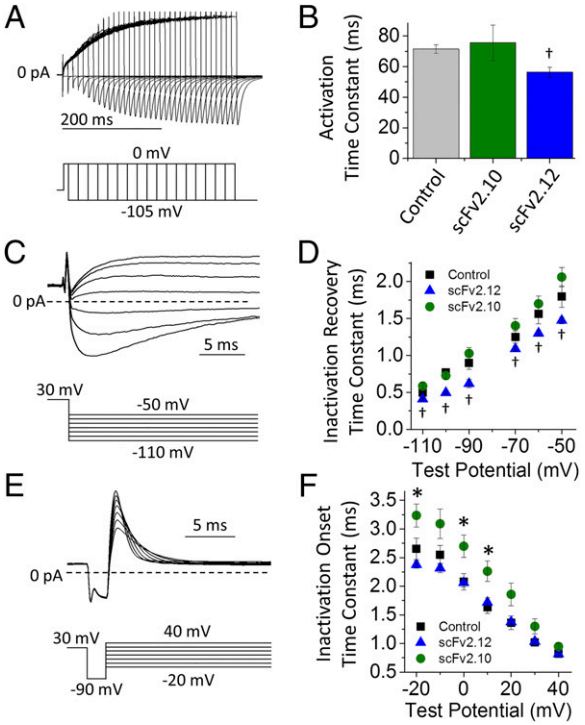

Fig. 5. scFv antibodies alter hERG 1a kinetics. Whole-cell patch clamp recordings from HEK293 cells expressing hERG 1a at 36 °C ± 1 °C. (*A*) Envelope of tails activation protocol and traces. (*B*) Activation time constant for control, 2.10, and 2.12 antibodies derived from peak tails as in *A*. (*C*) Inactivation recovery measured over a range of voltages at onset of voltage change from 30 mV. (*D*) Time constants of inactivation determined from single exponential fits to data as in *C* vs. test potential. (*E*) Inactivation onset measured with the three-pulse protocol over a range of potentials. (*F*) Inactivation time constants determined with single exponential fits to data as in *E* vs. test potentials. Data are means ± SEM ($n = 5$–10). *Statistical significance between scFv 2.10 and control at $P < 0.05$. †Statistical significance between scFv 2.12 and control at $P < 0.05$.

on gating kinetics is reflected in the exemplar traces in Fig. 6*E*. Current recorded from an untransfected HEK293 cell is shown in red in Fig. 6*E*. These results reflect how the differential binding of scFv 2.10 and scFv 2.12 to either the PAS-Cap or the PAS domain of hERG increases repolarizing charge during a ventricular AP.

scFv Antibodies Enhance I_{Kr} and Shorten AP Durations in Human Cardiomyocytes. We measured the effect of the antibodies on cardiac I_{Kr} from cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CMs) at physiological temperature (36 °C ± 1 °C). Similar to the effects on heterologously expressed hERG currents, scFv 2.10 and scFv 2.12 antibodies significantly increased steady-state I_{Kr} compared with control (Fig. 7 *A* and *B*), without a measurable effect on the conductance–voltage relationship of activation (Fig. 7*C*). Both antibodies also significantly increased currents evoked by AP voltage clamp (Fig. 7 *D* and *E*). Action potential durations (APDs) were correspondingly shortened as shown at 40% and 90% repolarization (Fig. 7 *F* and *G* and Table S4). Neither antibody affected the maximum diastolic potential, AP amplitude, or AP rise rate, suggesting the antibodies are specific for I_{Kr} (Table S4). Although with this approach we have not ruled out nonspecific effects of the antibody on other channels, the effects on AP morphology are consistent with a targeted increase in I_{Kr} as observed using patch clamp analysis in the cardiomyocyte and heterologous expression system. Overall, these data show that the PAS domains in native I_{Kr} channels are accessible to scFv 2.10 and scFv 2.12 antibody binding, allowing targeted enhancement of repolarizing I_{Kr} magnitude and subsequent shortening of the human cardiac AP.

Discussion

This work identifies two scFv antibodies (scFv 2.10 and scFv 2.12) that bind to different regions of the PAS domain and cause an increase in the total amount of current passing through the hERG channel in HEK293 cells and I_{Kr} in stem cell-derived human cardiomyocytes. At physiological temperatures, the effects of the scFv molecules on inactivation and activation dictate a net increase in current. The antibody that recognizes the globular PAS region (scFv 2.12) increases the rate of activation and accelerates the time course of inactivation recovery, effects that have been previously associated with the role of the PAS domain (19, 31). In contrast, the antibody that recognizes the PAS-Cap region (scFv 2.10) slows the time course of inactivation onset, an effect not previously associated with PAS-Cap function, which may reflect an allosteric effect of the antibody on the contiguous PAS domain.

The majority of characterized hERG channel modulators are small molecules, broadly divided into blockers and activators (or positive modulators). Blockers share a common binding site in the hERG pore domain cavity, blocking the ion permeation pathway of the channel, reducing the ion current associated with hERG, and often, leading to acquired LQTS (32, 33). In contrast, positive modulators of hERG bind at a pore domain site located between adjacent hERG subunits (34). The differences in chemical nature and mode of interaction with the channel (binding to the PAS domain) lead us to conclude that the two scFv molecules described form a different class of modulators of the hERG channel.

Our work with the scFv antibodies also reveals the potential of the cytosolic domains of hERG and, in particular, the PAS domain as targets for modulating hERG channel function. However, we have also recently shown the difficulties in identifying small molecules that bind to PAS domains from KCNH channels (27). From this perspective and despite the difficulties in targeting and delivering these molecules into specific cell cytosols, we must at least consider the therapeutic potential of antibodies targeting cytosolic domains. Although delivery of these molecules is a major obstacle at this time for their use as therapeutic agents, development of technologies for the cytosolic delivery of large protein molecules is an important focus of research (35–38), and it is reasonable to expect that, in the future, solutions to this problem will be found.

Comparison of the properties of the scFv molecules with other small molecule positive modulators shows that, despite binding to different regions, both the small molecules (39) and scFv molecules generally increase the magnitude of current associated with hERG by disrupting the voltage-dependent inactivation process and favoring the open state. However, the maximum effect of our scFv molecules is an approximately twofold increase in hERG or I_{Kr} currents, whereas the small molecule hERG-positive modulators have larger maximal effects that can lead to excessive APD shortening and arrhythmia at elevated doses (40). The subtler effect of scFv molecules targeted toward the hERG PAS domain could potentially correct LQTS without the risk of excessive shortening the QT interval and evoking arrhythmias. In this regard, scFv molecules targeting the PAS domain present a potentially safer mechanism of action.

Table 2. Time constants of activation, inactivation onset, and inactivation recovery

	Control, ms	scFv 2.10, ms	scFv 2.12, ms	*n*
Activation (0 mV)	71.5 ± 2.8	75.6 ± 11.5	56.3 ± 3.4*	5
Inactivation onset (10 mV)	1.6 ± 0.1	2.3 ± 0.2*	1.7 ± 0.1	5–8
Inactivation recovery (−50 mV)	1.8 ± 0.2	2.1 ± 0.1	1.5 ± 0.1*	5–10

All data are means ± SEM; *n* is the number of replicates.
*$P < 0.01$ compared with control.

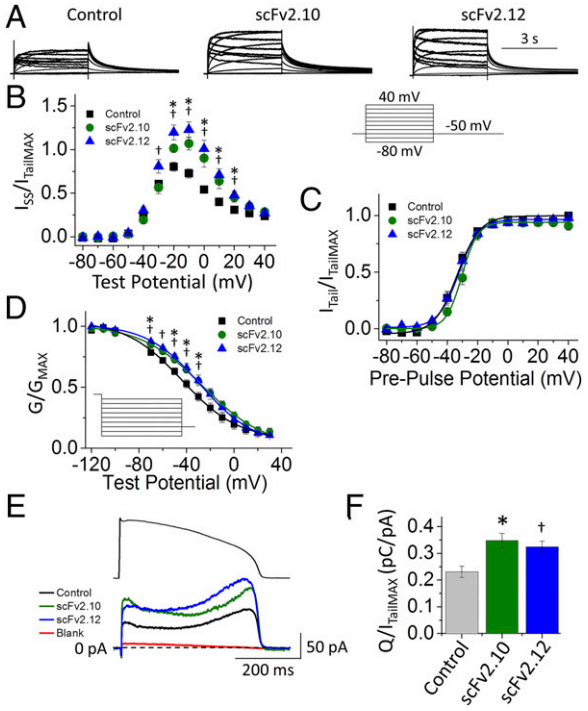

Fig. 6. scFv antibodies increase hERG 1a currents. (*A*) Traces from HEK293 cells expressing hERG 1a for vehicle control, scFv 2.10, and scFv 2.12 at 36 °C ± 1 °C. Pulse protocol is shown. (*B*) Relative steady-state current (I_{SS}) vs. test potential. (*C*) Normalized peak tail current vs. prepulse potential fitted with Boltzmann function for control (black), scFv 2.10 (green), and scFv 2.12 (blue). (*D*) Voltage dependence of inactivation for control (black), scFv 2.10 (green), and scFv 2.12 (blue). After a pulse to +40 mV, currents evoked over a range of potentials from −120 to 30 mV were measured. Normalized conductance vs. test potential fitted with a Boltzmann function (*Materials and Methods* and Fig. S4). (*E*) Currents from control (black), scFv 2.10 (green), scFv 2.12 (blue), and untransfected HEK cells (blank; red; *Lower*) during voltage command mimicking human ventricular AP (*Upper*). (*F*) Relative repolarizing charge for vehicle control (gray), scFv 2.10 (green), and scFv 2.12 (blue). Data are means ± SEM (n = 5–10). *Statistical significance between control and scFv 2.10 at $P < 0.05$. †Statistical significance between control and scFv 2.12 at $P < 0.05$.

Importantly, the scFv molecules have allowed us to gain insights into the properties of the PAS domain within the context of the full-length hERG channel. In particular, there is limited structural information about the PAS-Cap region, which is disordered in the structures of isolated PAS domains. The PAS-Cap is thought to extend away from the PAS domain and interact with the machinery influencing channel gating (17, 41, 42). Our functional data show that scFv 2.10 alters the properties of the hERG channel. By defining that scFv 2.10 interacts with the PAS-Cap and that the epitope involves residues R4 and R5, thought to be important for the interaction between PAS-Cap and the C linker of the hERG channel (41, 43), we have established that this channel region is not always engaged with the gating machinery. In at least one functional state of the channel, the N-terminal end of PAS-Cap releases from the gating machinery and becomes accessible for interaction with the scFv, a protein of 27 kDa. This finding fits well with the previously proposed model, in which the PAS-Cap domain transiently interacts with the C-terminal domain (41).

The scFv molecules also allowed us to explore the idea that channel PAS domains have a modulatory function. This idea has been supported by the role of PAS domains in other proteins and many studies that showed that mutations or deletions in the hERG PAS domain alter many aspects of channel gating (9, 15–18). However, those results simply indicate that the PAS domain is an important structural feature of KCNH channels but do not show the potential for allosteric modulation (28). With scFv 2.12, which binds to the globular region of the PAS domain, we have shown that interaction with another protein can modify the domain and elicit a functional change in the channel, establishing that it is possible to modulate hERG channel function allosterically through these cytosolic regions.

Strikingly, the functional impact of the scFv 2.12 antibody strongly resembles the functional differences observed between the heteromeric hERG 1a/1b channel and hERG 1a channel (19, 20). In hERG 1a/1b, there are fewer PAS domains present in the channel, resulting in faster deactivation but also, faster activation and recovery from inactivation, which in turn, yield larger currents. The observation of a similar functional phenotype with scFv 2.12 suggests that the antibody binds to PAS and changes the PAS interaction with its binding site in the channel, the CNBh domain. In effect, the antibody replicates the genetic modulation observed in the heteromeric channel as an allosteric modulation.

Materials and Methods

Protein Purification. hERG and mEAG1 PAS domain versions cloned with N-terminal GST or His tag were expressed and purified as previously described (12, 44).

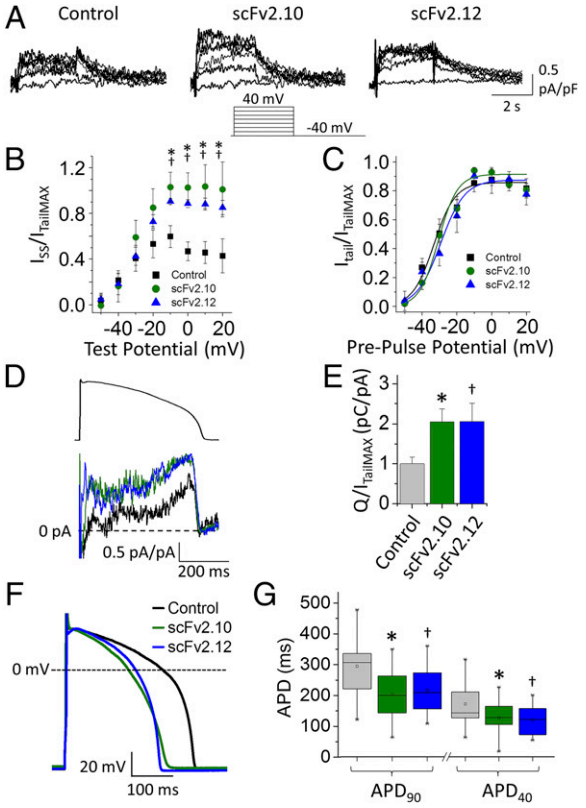

Fig. 7. scFv antibodies increase I_{Kr} and shorten APD. (*A*) Traces from iPSC-CMs for control, scFv 2.10, and scFv 2.12 at 36 °C ± 1 °C. Pulse protocol is shown. (*B*) Relative steady-state current vs. test potential. (*C*) Normalized peak tail current vs. prepulse potential fitted with Boltzmann function for control (black), scFv 2.10 (green), and scFv 2.12 (blue). (*D*) Currents evoked during AP voltage command (*Upper*) from control (black), scFv 2.10 (green), and scFv 2.12 (blue; *Lower*). (*E*) Relative repolarizing charge determined by integrating currents as in *D*. Data are means ± SEM (n = 9–12). (*F*) AP traces from control (black), scFv 2.10 (green), and scFv 2.12 (blue) Kir2.1-transduced iPSC-CMs. (*G*) Box plots of APDs measured at 90% and 40% repolarization times from control (black), scFv 2.10 (green), and scFv 2.12 (blue) Kir2.1-transduced iPSC-CMs. Boxes show 25 to 75% confidence intervals; error bars show 10 to 90% confidence intervals. The internal line is the median (n = 13–15). *Statistical significance between control and scFv 2.10 at $P < 0.05$. †Statistical significance between control and scFv 2.12 at $P < 0.05$.

Antibody Expression and Purification. scFv antibodies secreted to periplasmic space of *E. coli* BL21(DE3) from pComb3XSS vector or a modified pComb3XSS that includes a Thrombin cleavage site (30) were purified using His tag affinity and size exclusion chromatography (*SI Materials and Methods*).

Affinity Measurements. ITC experiments between scFv antibodies and hERG or mEAG1 PAS proteins were performed in MicroCal VP-ITC at 15 °C. hERG or mEAG1 PAS proteins at 200 μM solution were titrated into 20 μM antibody in the sample cell. Data were fitted using Origin 7 (MicroCal) (*SI Materials and Methods*).

GST Pulldown Assay. Purified GST protein or GST fusions of hERG and mEAG1 PAS domains (truncated or mutated) were bound to glutathione magnetic beads and incubated with purified scFv antibodies. After thorough washing, bound protein was eluted with SDS sample buffer. Bound scFv protein was detected by Western blot with anti-His antibody (*SI Materials and Methods*).

Electrophysiology. Patch clamp electrophysiology was carried out on HEK293 cells and human iPSC-CMs as described in detail in *SI Materials and Methods*.

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