Journal of Immunological Methods 375 (2012) 215–222

Contents lists available at SciVerse ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

The use of Bcl-2 over-expression to stabilize hybridomas specific to the HERG potassium channel

Jakub Sroubek [a], Yamini Krishnan [a], Jordan Chinai [a], Susan Buhl [b], Matthew D. Scharff [b], Thomas V. McDonald [a,c,*]

[a] Department of Molecular Pharmacology, Wilf Family Cardiovascular Research Institute, Albert Einstein College of Medicine, Bronx, NY 10461, United States
[b] Department of Cell Biology, Wilf Family Cardiovascular Research Institute, Albert Einstein College of Medicine, Bronx, NY 10461, United States
[c] Department of Medicine, Wilf Family Cardiovascular Research Institute, Albert Einstein College of Medicine, Bronx, NY 10461, United States

ARTICLE INFO

Article history:
Received 11 October 2011
Accepted 26 October 2011
Available online 15 November 2011

Keywords:
Hybridoma
Apoptosis
Bcl-2
HERG
Potassium channel

ABSTRACT

We encountered a high degree of clonal hybridoma loss in the course of generating antibodies specific for the hERG potassium channel. A protein that is crucial for controlling heart rhythm, is abundant in parts of the brain and is abnormally expressed in some tumors. Intracellular domains of the protein were used for immunogens and generated adequate antibody responses in mice. Subsequent hybridomas created using Ag8 myeloma fusion partner yielded clones that secreted specific antibody but none could be successfully maintained in culture. A variety of mechanisms, including polyploidy inherent to hybridoma development or production of cytotoxic antibodies, may be responsible for eventual loss of cell viability by mechanisms that may include apoptosis. When spleen cells were fused to the NSO myeloma cell line that stably over-expresses the anti-apoptotic protein Bcl-2, hybridoma clones were generated that remained viable in culture with high level of hERG-specific antibody production. When the parental NSO cell line not over-expressing Bcl-2 was used, no stable hybridomas were produced. Antibodies secreted by NSO-Bcl-2 hybridomas were specific for hERG and performed well in immunoblot, immunoprecipitation and immunofluorescence assays. This work demonstrates a feasible option when faced with antigens that seem to be associated with clonal instability in the process of generating monoclonal antibodies.

1. Introduction

One of the genes that most commonly harbors mutations leading to the hereditary cardiac long QT syndrome (LQTS) is HERG (human *Ether-a-go-go* related gene, also known as Kv11.1 and KCNH2). HERG encodes an intrinsic surface membrane protein that forms a potassium ion-selective channel and is expressed in the heart, brain and several other peripheral tissues. Deleterious mutations of HERG are associated with cardiac rhythm disturbances that may lead to syncope and sudden death at a young age (Moss, 2003; Modell and Lehmann, 2006; Sanguinetti et al., 1995). More than 300 LQTS-associated mutations have been reported and of those that have been studied, more than half show assembly, trafficking and protein stability defects (Anderson et al., 2006). Moreover, acquired LQTS – a much more common entity – is nearly always due to drug interactions with the HERG channel that either block its ion conduction properties or confer trafficking defects upon the wild type channel (Witchel, 2010). Such common drug interactions have led both the U.S. Food and Drug Administration as well as EU's European Medicines Agency to mandate that every newly developed drug be tested for such an adverse effect (1996; 2005; Darpo et al., 2006). Accordingly, high-specificity and affinity reagents such as monoclonal antibodies are desirable to enhance future investigative efforts. We set out to develop HERG monoclonal antibodies that could potentially be used for these applications. Unexpectedly, we encountered

* Corresponding author at: 1300 Morris Park Ave, F-G35, Bronx, NY 10461, United States. Fax: +1 718 430 8989.
E-mail address: tom.mcdonald@einstein.yu.edu (T.V. McDonald).

0022-1759/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jim.2011.10.014

repeated difficulty in keeping HERG-specific antibody-producing hybridomas alive in stark contrast to simultaneous creation of monoclonal lines against another potassium channel protein. Here report the nature of the problem we encountered and describe one possible solution that we found successful.

2. Methods

2.1. Antigen preparation

Small antigen peptides were prepared first by performing PCR on HERG cDNA template (see Fig. 1 A) using the following primer pairs: F2: cggaattccagagccgtaagttcatcatcgc and cgaagcttttacccatcctcgttcttcacgg, F4: cggaattcgctgtcatcatgttcatcc and cgaagctttagttgtccatggctgtcac, F6: cggaattcgtgacagccatggacaaccac and cgaagcttttacgaggtggagttgagcaag, F8: cggaattcgactccgacctcgtgcgctac and cgaagctttagatggtccagcggtggatgc, F10: cggaattcaacatggagcagcc and cgaagcttttagatcttctctgagttggtgttg, F12: cggaattcgcccgctaccacacacagatg and cgaagctttacaccagtgtgtcccctggc, F14: cggaattccatgctggggacctgctc and cgaagcttttagcccgggatcatgttgg, F16: cggaattctcccccggcagtacg and cgaagcttttagttgcaagtgtcgctgctc, and F18: cggaattcgcgtgtgaggagctgcccc and cgaagcttttaactgcccgggtccgagccg. The PCR products were digested with EcoRI and HindIII, and ligated into the pMAL-c2 vector (New England BioLabs). These constructs encoding maltose binding protein (MBP)-HERG fragment fusions were expressed in Bl21(DE3) *E. coli* strain (Invitrogen). Recombinant protein-expressing bacteria were pelleted, resuspended in MBP buffer (20 mM Tris–HCl pH ~ 7.4, 0.2 M NaCl, 10 mM beta-mercaptoethanol, 1 mM EDTA) and supplemented with complete protease inhibitors (Roche). After cell disruption, protein lysates were applied to an amylose resin (New England BioLabs) affinity column and washed. Elution was accomplished using MBP buffer containing 10 mM maltose. Final purification was achieved using FPLC gel filtration.

2.2. SDS page and immunoblots

Antigen samples (F2–F18) were separated on 4–15% gradient gels (BioRad) and stained with Coomassie brilliant blue dye. HEK293 cell lysates were separated on 7.5% linear gels and transferred onto nitrocellulose membranes via a semi-dry blotting unit (Fischer Scientific). The membranes were blocked with 5% non-fat milk, probed with appropriate primary antibody and then incubated with either IRDye700 donkey-anti-rabbit or IRDye800 donkey-anti-mouse (Rockland) secondary antibodies. Infrared signal was scanned using the Odyssey system (LiCor).

2.3. Immunization

6-week old female BALB/c mice were used and all procedures and handling were in accordance with the approved protocol reviewed by the Einstein Animal Institute Committee. Mice were immunized with the following pools of antigen: pool A: F2, F4, and F8; pool B: F10; pool C: F12, F14, F16, and F18 (Fig. 1A). All immunizations were via intraperitoneal injection. Initial immunization on day-1 was done using an emulsion of 100 µl of complete Freund's adjuvant (CFA) mixed with 100 µl of antigen pool per mouse. Subsequent boosts on week 3 and week 6 were similar, except incomplete Freund's adjuvant (IFA) was used. Anti-HERG antibody titers were monitored from tail-bled sera, using a 28-channel miniblotter cartridge (Immunetics) on nitrocellulose membranes carrying single-lane HEK293-HERG lysate.

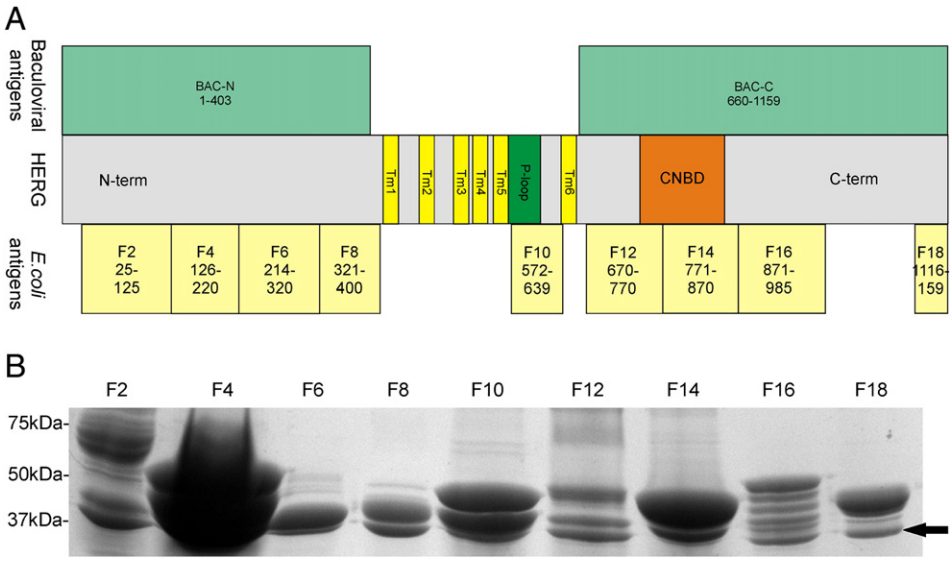

Fig. 1. Antigen design and expression. A) Diagram of HERG antigen peptides. Longer antigens (BAC-N and BAC-C) were expressed in baculoviral system as GST fusions. Shorter antigens (F2-14) were expressed in *E. coli* as MBP fusions. B) Coomassie brilliant blue stain of purified HERG antigens expressed in *E. coli*. Baculoviral constructs achieved very poor expression and are not shown. Putative MBP-only degradation/truncation product is marked by an arrow.