Engineering KRAS Mutation-Specific Affinity Binders: A New Frontier in Cancer Research

Poster #LB060

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Abstract

Cancer involves genetic and proteomic changes. Analyzing cellular shifts from normal to cancerous states is vital. The KRAS gene, often mutated at glycine codon 12, plays a key role in many cancers, including pancreatic, lung, and colon. While recent advances have targeted the G12C variant with small molecules, the more prevalent G12D and G12V mutations have no specific treatments. We are developing KRAS mutant-specific binders through novel immunization and screening to target wild-type and various mutations like G12D, G12S, G12C, G12A, G12R, and G12V for research on cancer pathways and cell organization. These binders may also be valuable for prognosis and therapy. Thorough evaluation of these tools is essential for their integration into laboratory and clinical practice.

Introduction

Site-directed antibody (Ab) development employs B cell cloning to isolate specific B cells for monoclonal antibody (mAb) production. Starting with peripheral blood mononuclear cells (PBMCs) from whole blood, cells are cultured in 384-well plates to allow B cell proliferation and antibody expression. Over six days, an ELISA screens for desired mAbs. Positive ELISA results lead to sub-cloning and expression in ExpiCHO cells. After identification, site-directed mAbs are characterized to confirm their specificity and function. Purified mAbs are tested by Western blot against wild-type proteins and variants, standardized to 1 µg/mL, with a secondary antibody-only blot and maltose-binding protein as negative controls to eliminate non-specific binding.

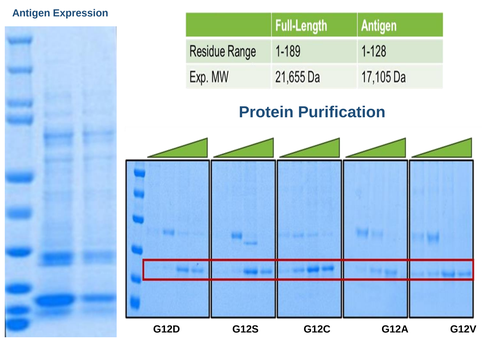
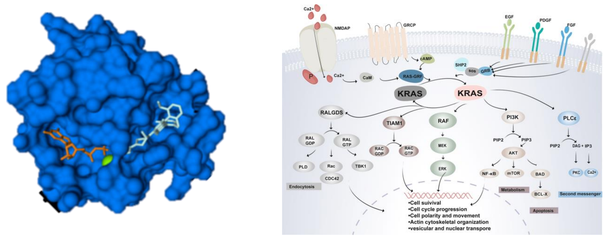


Figure 2. KRAS protein expression and purification. From transformed bacteria resulted in >90% pure and soluble KRAS for all mutant and wild type proteins.



[from: https://www.nature.com/articles/s41392-021-00780-4]

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HRAS        MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
NRAS        MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
KRAS4A      MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
KRAS4B      MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
            ************************************************************

HRAS        QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHQYREQIKRVKDSDDVPMVLVGNKCDL
NRAS        QEEYSAMRDQYMRTGEGFLCVFAINNSKSFADINLYREQIKRVKDSDDVPMVLVGNKCDL
KRAS4A      QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDL
KRAS4B      QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDL
            ********************************:*** **:**********:**********

HRAS        AARTVESRQAQDLARSYGIPYIETSAKTRQGVEDAFYTLVREIRQHKLRKLNPPDESGPG
NRAS        PTRTVDTKQAHELAKSYGIPFIETSAKTRQGVEDAFYTLVREIRQYRMKKLNSSDDGTQG
KRAS4A      PSRTVDTKQAQDLARSYGIPFIETSAKTRQRVEDAFYTLVREIRQYRLKKISKEE-KTPG
KRAS4B      PSRTVDTKQAQDLARSYGIPFIETSAKTRQGVDDAFYTLVREIRKHKEKM-SKDG-KKKK
            :***::**:**:******:********** *:**********:::  :   .

HRAS        CMSCK-CVLS  189
NRAS        CMGLP-CVVM  189
KRAS4A      CVKIKKCIIM  189
KRAS4B      KKSKTKCVIM  188
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Figure 1. KRAS structure and sequences. (Left) KRAS structure Highlighting the GTPase binding site. (**Middle) KRAS pathway.** KRAS is a molecule that needs to attach to cell membranes through specific modifications to function. It switches between active and inactive states, influenced by different proteins that either activate or inactivate it. Enzymes like farnesyltransferase help it attach to the membrane, while others ensure it's correctly positioned to relay signals inside the cell, influencing cell behavior. ***(Right)* KRAS protein sequence.** KRAS, involved in cell signaling, is composed of 188/189 amino acids across three regions with distinct homology and functions. The first 85 amino acids, essential for its structural and functional integrity, are highly conserved. The next 80 amino acids share 85% similarity across human RAS proteins, supporting guanine nucleotide binding and signaling. The last section, with only 8% homology, permits KRAS's involvement in various signaling pathways

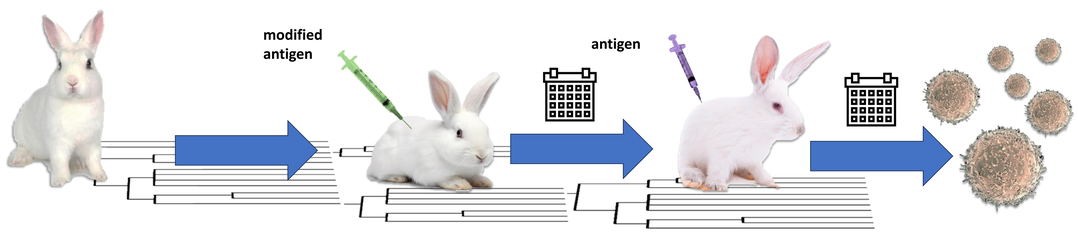

Figure 3. The Epivolve™ method Epivolve uses PTMs to enhance immune recognition in diseases like cancer and autoimmunity. Through B cell somatic hypermutation (SHM), it increases antibody diversity for detecting PTM-modified and unaltered amino acids, referred to as Epitope Spreading and Degeneracy. Affinity maturation further improves the binding between antibodies and antigens, ensuring selection of B cells producing high-affinity antibodies. This methodology fine-tunes antibodies to identify the antigen's native conformation, even after initial PTM binding.

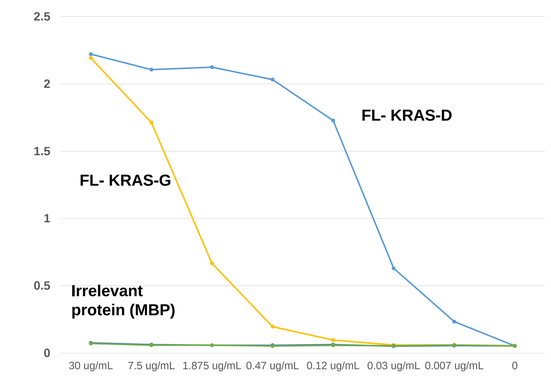

Figure 4. ELISA Analysis against full length KRAS. Epivolve employs a synthetic amino acid called "mod1" as a substitute for certain native residues in peptides or proteins, serving as a pseudo-hapten to bolster the selection of agents with specificity for this modification. Antibodies that bind to "mod1" are then evolved to also identify the native antigen sequence

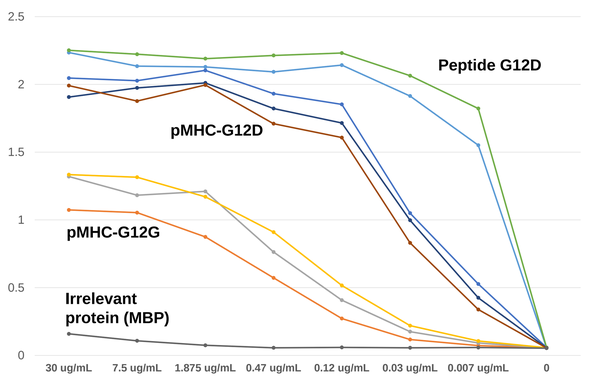

Figure 5. ELISA against KRAS G12D pMHC. Epivolve employs a synthetic amino acid called "mod1" as a substitute for certain native residues in peptides or proteins, serving as a pseudo-hapten to bolster the selection of agents with specificity for this modification. Antibodies that bind to the MHC have been identified, enhancing the distinction and specificity of affinity agents.

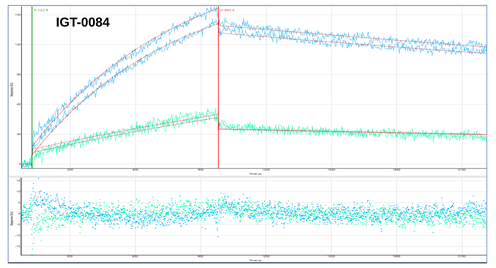

Figure 6. IGT-0084 binding kinetics vs KRAS G12D. Surface-attached antibodies at 50 µg/ml facilitated kinetic analysis via LSA Kinetic software, with two G12D antigen concentrations showing association and dissociation rates, ka = 2.7×10^3 M^-1s^-1 and kd = 1.4×10^-4 s^-1, respectively, and a 50 nM affinity constant (KD). No specific binding was observed for KRAS antigens with G12G or mutations G12S, G12C, G12R, and G12A.

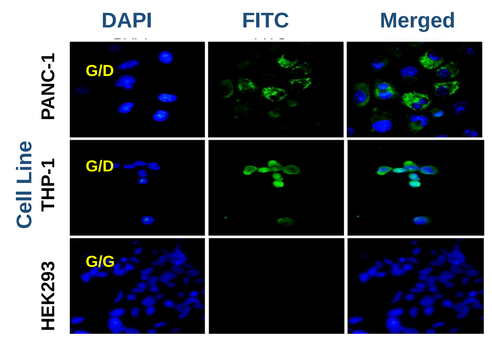

Figure 7. IGT-0084 Immunocytochemistry. Imaging results for IGT-0084 G12D recAb on PANC-1 (KRAS G12 Heterozygous), THP-1 (NRAS G12D Heterozygous) and HEK293 KRAS WT. Methanol fixed cells were probed with recAb at 1/50 dilution (20ug/ml) and visualized using anti Rabbit AF488.

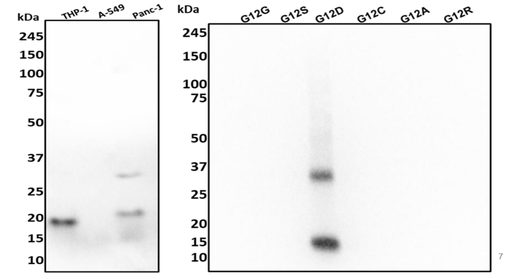

Figure 8. IGT-0084 Western. 40 µg of whole cell lysate or 500 ng of *in vitro* expressed G12 WT and mutants were probed with IGT-0084, the KRAS G12D recAb, at 3 µg/ml

Discussion and Summary

Epivolve's innovation leverages epitope spreading and degeneracy, alongside affinity maturation, to enhance an animal's natural immune defenses. This strategy broadens the immune response to specific antigens, which is beneficial for treating diseases requiring strong immune recognition and response. It presents a viable strategy for creating vaccines and immunotherapies capable of targeting a spectrum of antigens. Such advancements hold significant promise for personalized medicine applications in oncology, autoimmune disorders, and infectious diseases.

- G12 KRAS mutation drives key cancers.
- Epivolve pinpoints amino acid-specific KRAS antibodies.
- High-affinity antibody identified for KRAS G12D.
- IGT-0084 clone targets G12D KRAS, excludes others.
- Platform adaptable for varied genetic and protein modifications.