Discovery and Characterization of Epitope-Diverse Mismatch Repair (MMR) Protein Antibodies

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Abstract and Introduction

Huntington's disease (HD) is a devastating neurodegenerative disorder driven by an inherited CAG trinucleotide repeat expansion (≥36) in the huntingtin gene. Genome- and transcriptome-wide association studies (G/TWAS) have illuminated the DNA mismatch repair (MMR) pathway components as critical modifiers of disease progression, including onset age of motoric symptoms. By fueling somatic instability and repeat expansion, MMR activity may hold the key to understanding—and ultimately altering—the trajectory of HD. To enable interrogation of this mechanism, we have developed a suite of highly-specific, high-affinity monoclonal antibodies (mAbs) targeting FAN1, MLH1, MLH3, MSH2, MSH3, PMS1, and PMS2. These human/rodent cross-reactive mAbs exhibit remarkable sequence diversity and recognize 5–12 distinct epitopes per target, providing new reagents to probe MMR biology. This next-generation antibody toolkit provides much needed reagents to develop sensitive immunoassays to quantitate and visualize the MMR proteins in biofluids, cells and tissues.

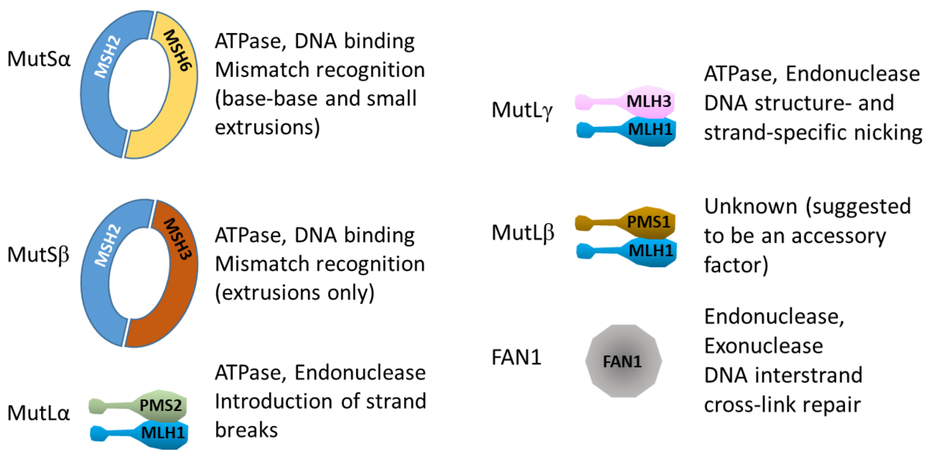

Figure 1. MMR proteins. We sought to efficiently identify a panel of diverse antibodies that recognize various epitopes of native MMR proteins in their native complexes. Many MMR proteins pair to form distinct endogenous heterodimers that share common components (Iyer et al., Goold et al.)

Target	Recombinant Lead Production	Hu/rodent x-reactive	Epitope Bins	Affinity (KD)	Potency (EC50) nM
MSH3	19	11	6	14 pM to 14 nM	0.35 to 17.31
MSH2	20	18	5	0.12 to 40 nM	0.38 to 12.07
PMS2	11	7	9	all <1 nM	0.28 to 4.04
MLH1	12	9	5	17 pM to 1.1 nM	0.27 to 3.0
PMS1	12	10	5	10 pM to 1.7 nM	0.33 to 20.51
MLH3	18	11	8	8.5 pM to 740 pM	0.13 to 11.36
FAN1	12	9	12	40 pM to 88 nM	0.41 to 86

Table 1. Summary of MMR mAb Discovery. For each MMR target, from left-to-right are first the number of lead mAbs expressed using the TunaCHO® platform (all formatted as mIgG1); the number of human/rodent cross-reactive mAbs among those selected for recombinant production; the number of epitope bins represented in the set of selected mAbs; the range of binding affinities (K_D) by Carterra® arrayed SPR; and binding potency (EC_{50}) by ELISA. The antigens used for each target with shared components were: MSH2 (MutSα); MLH1 (MutLα); and MLH3 (MutLγ).

Methods

For each MMR target, 11-20 mAbs were selected for recombinant production using Curia's TunaCHO® platform. Binding affinity (K_D) was determined by Carterra® arrayed SPR. An anti-mouse Fc lawn was used to capture antibodies; the target was then added and capture kinetics run for 5 minutes of association and 15 min of dissociation. Binding potency (EC_{50}) was determined by ELISA. Epitope binning was performed by biolayer interferometry (BLI) or pairwise ELISA using unlabeled capture mAbs and biotinylated detection antibodies.

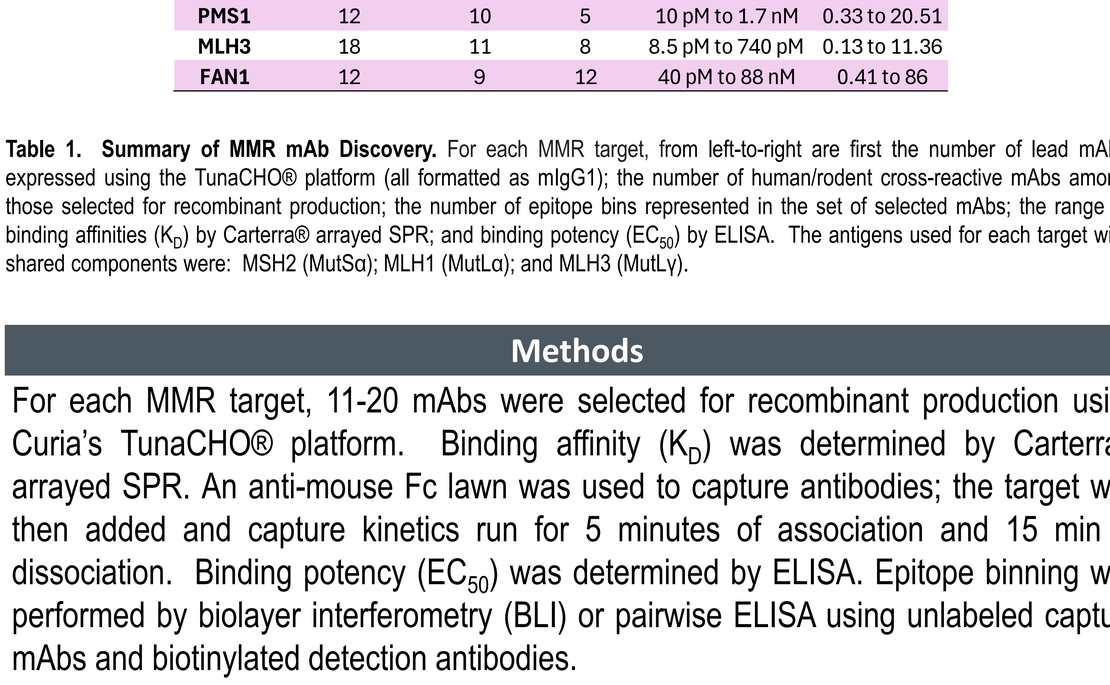

Figure 2. Epitope binning. For each MMR target, epitope binning was performed against the indicated antigen by assessing pairwise competition binding data and assigning bins using Carterra Epitope™ Software. Pairwise competition data was generated by biolayer interferometry (BLI) for all targets except MLH3, for which sandwich ELISA was used. The epitope bin assignments are color coded for each MMR target. Solid lines indicate symmetric blocking; dashed lines indicate asymmetric blocking. Larger spatial distances between bins reflect greater dissimilarity in their blocking/binding profiles.

Results and Discussion

Following PentaMice® platform immunizations and hybridoma-based antibody discovery, we obtained sequences for >100 mAbs that bind target MMR proteins (**Fig. 1**) with high affinity and potency (**Table 1**). For each target, we identified mAbs that bind between 5 and 12 distinct epitopes (**Fig. 2**). The mAbs utilize a diverse set of V and J gene alleles and have a high degree of sequence diversity in the V_H/V_L CDR3 domains. The mAbs have good *in silico* developability features (low Z^2 scores) on par with 20 approved and successfully manufactured therapeutic mAbs; and good yields from recombinant TunaCHO® productions (~8 mg from 100 mL). Greater than 86% of the over 100 mAbs retained similar binding affinities (K_D within 3-fold) when produced recombinantly as mIgG1 compared to their hybridoma purified mAb counterparts, demonstrating the high quality of Curia's discovery, engineering, production, and characterization workflow.

For MSH3, we identified antibody binding domains for our newly discovered mAbs by combining MSH3 domain-deletion mutant ELISA binding data with epitope binning data. mAb binding was mapped onto the predicted MSH3 structure (**Fig. 3**). To evaluate the MSH3 and MSH2 mAbs for potential utility in biomarker detection, we next assessed the ability of the mAbs to work in pairs in sandwich ELISAs. We identified 5 unique pairs of MSH3-specific mAbs that detect MSH3 in MutSβ and 10 unique pairs of MSH2-specific mAbs that detect MSH2 in MutSα (**Fig. 4**).

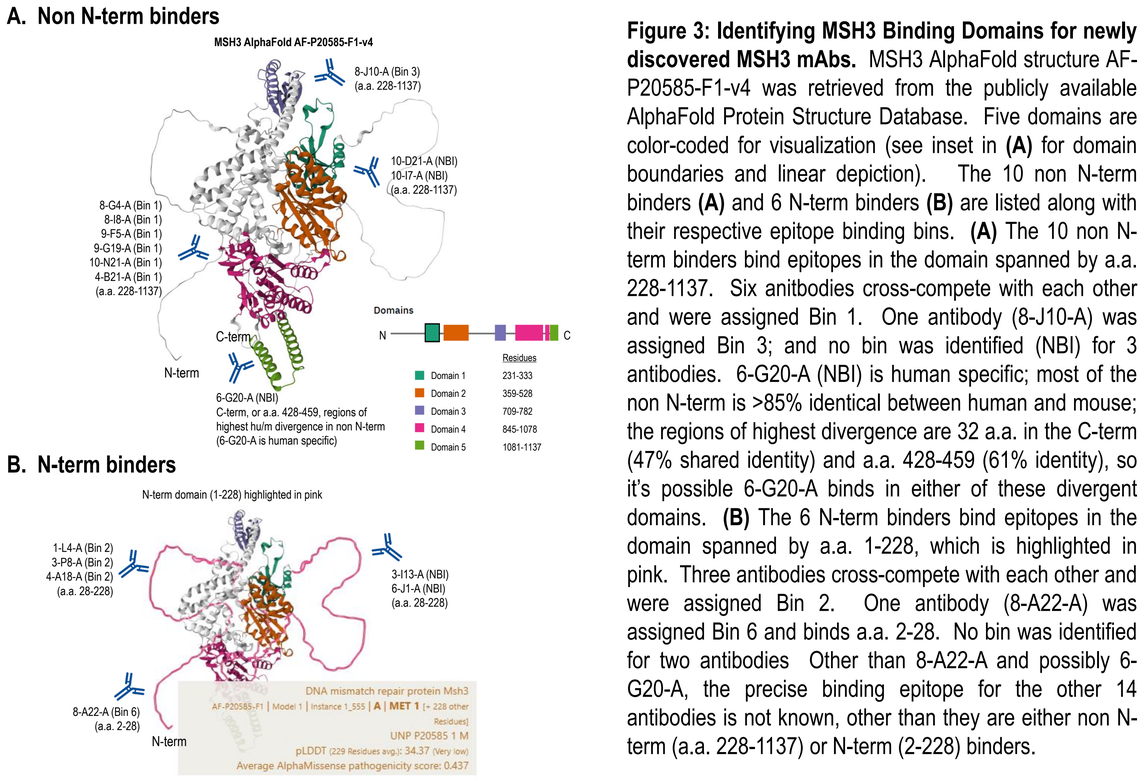

Figure 3: Identifying MSH3 Binding Domains for newly discovered MSH3 mAbs. MSH3 AlphaFold structure AF-P20585-F1-v4 was retrieved from the publicly available AlphaFold Protein Structure Database. Five domains are color-coded for visualization (see inset in **(A)** for domain boundaries and linear depiction). The 10 non N-term binders **(A)** and 6 N-term binders **(B)** are listed along with their respective epitope binding bins. **(A)** The 10 non N-term binders bind epitopes in the domain spanned by a.a. 228-1137. Six anitbodies cross-compete with each other and were assigned Bin 1. One antibody (8-J10-A) was assigned Bin 3; and no bin was identified (NBI) for 3 antibodies. 6-G20-A (NBI) is human specific; most of the non N-term is >85% identical between human and mouse; the regions of highest divergence are 32 a.a. in the C-term (47% shared identity) and a.a. 428-459 (61% identity), so it's possible 6-G20-A binds in either of these divergent domains. **(B)** The 6 N-term binders bind epitopes in the domain spanned by a.a. 1-228, which is highlighted in pink. Three antibodies cross-compete with each other and were assigned Bin 2. One antibody (8-A22-A) was assigned Bin 6 and binds a.a. 2-28. No bin was identified for two antibodies Other than 8-A22-A and possibly 6-G20-A, the precise binding epitope for the other 14 antibodies is not known, other than they are either non N-term (a.a. 228-1137) or N-term (2-228) binders.

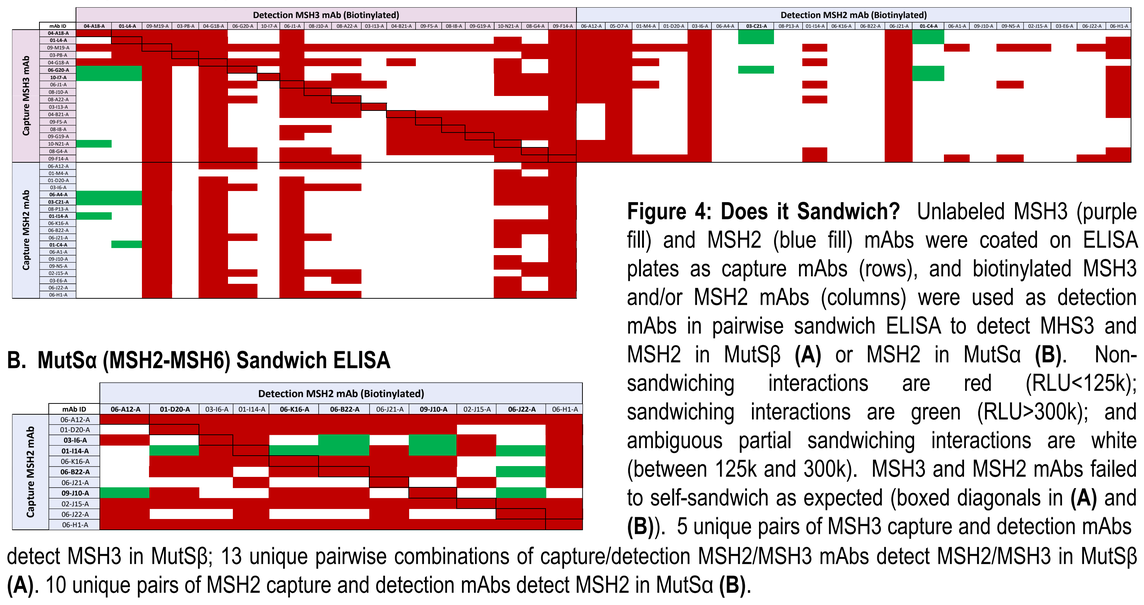

Figure 4: Does it Sandwich? Unlabeled MSH3 (purple fill) and MSH2 (blue fill) mAbs were coated on ELISA plates as capture mAbs (rows), and biotinylated MSH3 and/or MSH2 mAbs (columns) were used as detection mAbs in pairwise sandwich ELISA to detect MHS3 and MSH2 in MutSβ **(A)** or MSH2 in MutSα **(B)**. Non-sandwiching interactions are red (RLU<125k); sandwiching interactions are green (RLU>300k); and ambiguous partial sandwiching interactions are white (between 125k and 300k). MSH3 and MSH2 mAbs failed to self-sandwich as expected (boxed diagonals in **(A)** and **(B)**). 5 unique pairs of MSH3 capture and detection mAbs detect MSH3 in MutSβ; 13 unique pairwise combinations of capture/detection MSH2/MSH3 mAbs detect MSH2/MSH3 in MutSβ **(A)**. 10 unique pairs of MSH2 capture and detection mAbs detect MSH2 in MutSα **(B)**.

Discussion and Conclusions

These high-affinity, epitope-diverse mAbs provide a powerful toolkit to dissect the role of DNA mismatch repair proteins in HD. By enabling precise interrogation of MMR-driven somatic instability, they lay the foundation for mechanistic insights and future diagnostic/therapeutic strategies. In particular, the discovery of pairs of mAbs suitable for sandwich capture/detection methods for MSH3 and MSH2 opens the door for biomarker quantification of these key DNA mismatch repair proteins. By enabling precise, high-sensitivity detection of MSH3 and MSH2 in biosamples, it will be possible to track and correlate their levels with the rate of somatic CAG expansion in HD, and to assess the efficacy of MMR- or huntingtin protein-targeted therapeutics designed to slow disease progression.

References

1. Iyer, Ravi R., and Anna Pluciennik. "DNA mismatch repair and its role in Huntington's disease." *Journal of Huntington's Disease* 10.1 (2021): 75-94.