Cross-Reactivity Studies of Human and Mouse Tissues Using Affinity Purified Polyclonal Anti-Human Antibodies

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Abstract

An ongoing initiative by the Swedish Human Protein Atlas (HPA) program (www.proteinatlas.org) constitutes unraveling the human proteome by systematically mapping protein expression in different human tissues. The HPA program mainly strives to provide information to facilitate research within Academia and industry. This goal is accomplished by generating quality controlled HPA Antibodies against all human proteins. The HPA Antibodies are designed to have high specificity and to work in various application platforms. Unique antigens called Protein Epitope Signature Tags (PrESTs) are protein stretches of 70-150 amino acids in length, each representing a specific protein in the human proteome. Polyclonal antibodies raised against these PrESTs are purified according to a stringent two-step affinity purification procedure, which generates highly specific polyclonal antibodies also dubbed mono-specific antibodies (msAbs).

In this study, a set of HPA Antibodies have been selected in order to investigate whether msAbs, showing low cross-reactivity with non-target proteins in general, can be successfully used when studying the target protein counterpart in mouse. The msAbs were tested in immunostaining techniques such as immunohistochemistry (IHC) and Western blot (WB) on both human and mouse tissues to determine protein localization, distribution and proper target molecular weight(s). Normal human and mouse tissues from 21 histological compartments were represented in tissue microarrays and evaluated by enzyme based IHC. In addition, a panel of 5 different tissue lysates were evaluated by WB.

We hereby demonstrate by IHC and WB, that the majority of the tested msAbs show high specificity towards analog proteins in human and mouse, despite less than 100% similarity in the binding sequence of the proteins. This may reflect an advantage for the msAbs, explained by multi-epitope recognition, in contrast to e.g. monoclonal antibodies.

Introduction

To determine inter-species cross-reactivity, 84 HPA Antibodies with validated specificity to human proteins were analyzed by IHC in 21 different tissues from both human and mouse. In addition, 10 antibodies were further tested in a WB panel representing 5 different tissues. The HPA Antibodies are designed and titrated against human proteins and no further titration against mouse proteins has been performed. Thus, the same IHC protocols were used for both species. Evaluation of staining distributions and intensities were done by visual examination.

(Protocols for IHC and WB procedures are available at www.atlasantibodies.com)

Results

We define cross-reactivity between mouse and human as a high similarity in the intracellular IHC staining pattern. Differences at the histological level are expected for various reasons and was accepted to some extent.

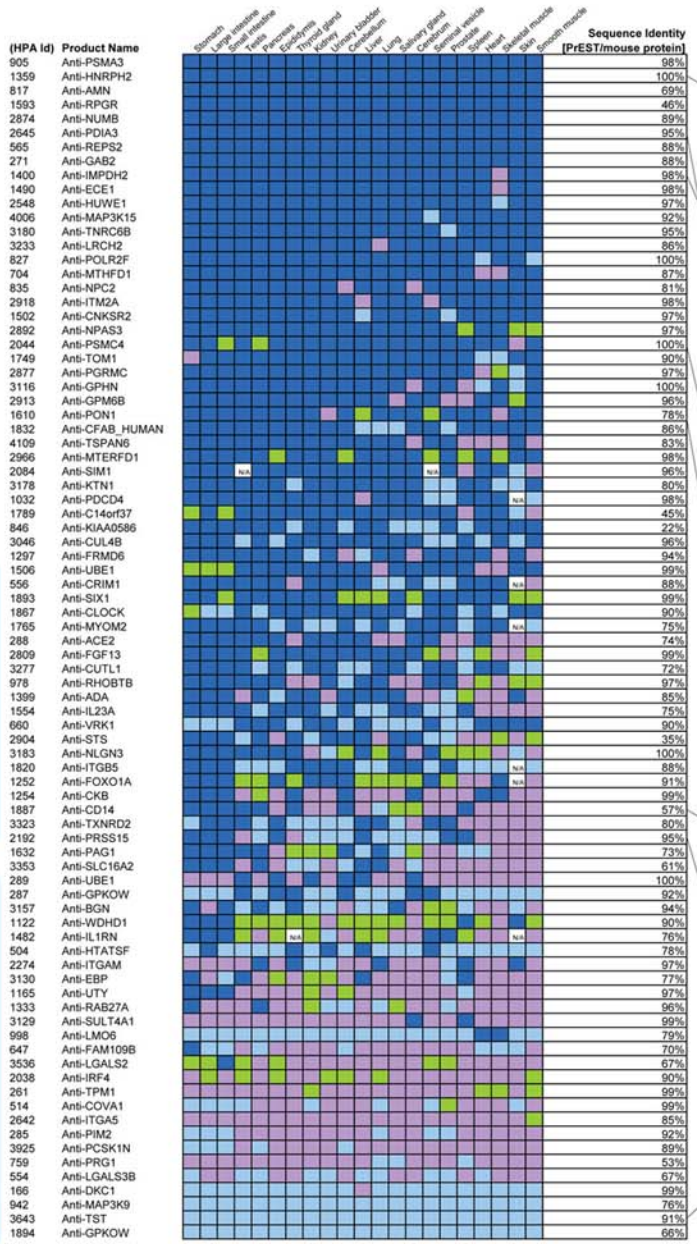
The inter-species comparison is made based on the validated intracellular staining distribution observed in human. Mouse staining is then evaluated accordingly.

IHC-tabel

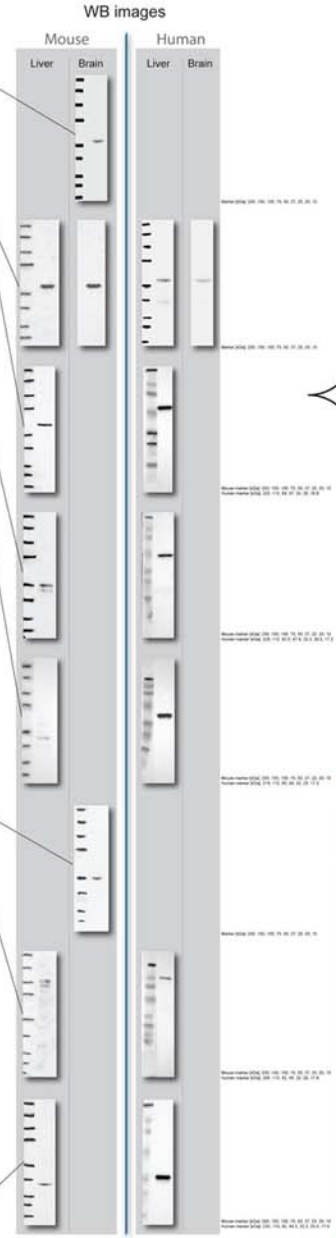
Color code of staining results:

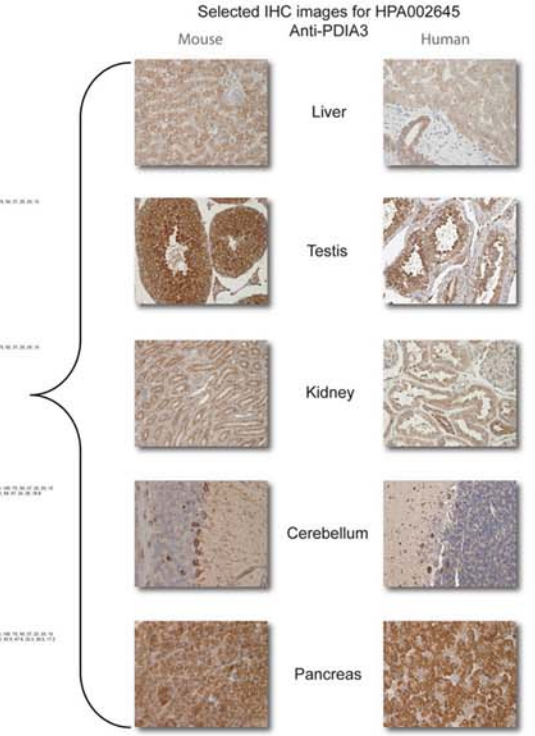
- Positive in mouse and human
- Negative in mouse and human
- Negative in mouse, positive in human
- Positive in mouse, negative in human

Positive - Same intracellular distribution in mouse and human
Negative - No staining in any cells



(HPA Id)	Product Name	Sequence Identity [PrEST/mouse protein]
905	Anti-PSMA3	98%
1359	Anti-HNRPH2	100%
817	Anti-AMN	69%
1593	Anti-RPGR	46%
2874	Anti-NUMB	89%
2645	Anti-PDIA3	95%
565	Anti-REPS2	88%
271	Anti-GAB2	88%
1400	Anti-IMPDH2	98%
1490	Anti-ECE1	98%
2548	Anti-HUWE1	97%
4006	Anti-MAP3K15	92%
3180	Anti-TNRC6B	95%
3233	Anti-LRCH2	86%
827	Anti-POLR2F	100%
704	Anti-MTHFD1	87%
835	Anti-NPC2	81%
2918	Anti-ITM2A	98%
1502	Anti-CNKSR2	97%
2892	Anti-NPAS3	97%
2044	Anti-PSMC4	100%
1749	Anti-TOM1	90%
2877	Anti-PGRMC	97%
3116	Anti-GPHN	100%
2913	Anti-GPM6B	96%
1610	Anti-PON1	78%
1832	Anti-CFAB_HUMAN	86%
4109	Anti-TSPAN6	83%
2966	Anti-MTERFD1	98%
2084	Anti-SIM1	96%
3178	Anti-KTN1	80%
1032	Anti-PDCD4	98%
1789	Anti-C14orf37	45%
846	Anti-KIAA0586	22%
3046	Anti-CUL4B	96%
1297	Anti-FRMD6	94%
1506	Anti-UBE1	99%
556	Anti-CRIM1	88%
1893	Anti-SIX1	99%
1867	Anti-CLOCK	90%
1765	Anti-MYOM2	75%
288	Anti-ACE2	74%
2809	Anti-FGF13	99%
3277	Anti-CUTL1	72%
978	Anti-RHOBTB	97%
1399	Anti-ADA	85%
1554	Anti-IL23A	75%
660	Anti-VRK1	90%
2904	Anti-STS	35%
3183	Anti-NLGN3	100%
1820	Anti-ITGB5	88%
1252	Anti-FOXO1A	91%
1254	Anti-CKB	99%
1887	Anti-CD14	57%
3323	Anti-TXNRD2	80%
2192	Anti-PRSS15	95%
1632	Anti-PAG1	73%
3353	Anti-SLC16A2	61%
289	Anti-UBE1	100%
287	Anti-GPKOW	92%
3157	Anti-BGN	94%
1122	Anti-WDHD1	90%
1482	Anti-IL1RN	76%
504	Anti-HTATSF	78%
2274	Anti-ITGAM	97%
3130	Anti-EBP	77%
1165	Anti-UTY	97%
1333	Anti-RAB27A	96%
3129	Anti-SULT4A1	99%
998	Anti-LMO6	79%
647	Anti-FAM109B	70%
3536	Anti-LGALS2	67%
2038	Anti-IRF4	90%
261	Anti-TPM1	99%
514	Anti-COVA1	99%
2642	Anti-ITGA5	85%
285	Anti-PIM2	92%
3925	Anti-PCSK1N	89%
759	Anti-PRG1	53%
554	Anti-LGALS3B	67%
166	Anti-DKC1	99%
942	Anti-MAP3K9	76%
3643	Anti-TST	91%
1894	Anti-GPKOW	66%





Conclusions

In this study, cross-reactivity by IHC is defined as high similarity in intracellular distribution between human and mouse. Differences in the histological distribution are accepted to some extent.

IHC staining in mouse tissues was generally stronger than in human tissues. A possible explanation may be that the mice analyzed are usually young and vital, whereas human samples originate from older individuals. Also, the mouse tissues are more stringently histo-processed compared to human surgical material, e. g. mouse material is in general more rapidly placed in fixative. Consequently, the difference in staining intensity may hamper the comparison of the staining results in human and mouse tissues.

Earlier experiments have shown that approximately one third of the HPA Antibodies have been successfully tested in both WB and IHC. We show that 8 out of 10 selected antibodies were succefully tested on both mouse and human tissues in WB and IHC. Cross-reactivity in WB was predominately observed in liver tissues.

It can finally be concluded that percentage sequence identity between PrEST protein and corresponding mouse protein fragment cannot be correlated to any cross-reactivity in the present applications.

Our study demonstrate that 95% of the tested antibodies show the same reactivity in both species. This clearly reflects the versatility of affinity purified polyclonal HPA Antibodies.