ATLAS ANTIBODIES

Exploring Proteins with Unknown Function using Triple A Polyclonals

Creation of a Whole Genome-based Human Protein Atlas

The human genome sequence was first published in 2001 and an ambitious effort was initiated 2003 to create a comprehensive human protein atlas based on the genome sequence. This effort resulted in an impressive resource of human protein expression profiles in a multitude of normal tissues, cancer cells and cell lines with validated Triple A Polyclonals as reagents. April 2013, 15,100 human proteins have been analyzed within this effort and the results are publicly available on the Human Protein Atlas (HPA) web portal (proteinatlas.org)[1,2,3]. Each year protein expression and localization data of approximately 2,000 new proteins are added to the portal. By 2015, a first draft of the localization of the full human proteome will be ready.

Antibodies for Proteins with Unknown Function

Triple A Polyclonals are unique in the way they are developed by a strategy originating from genome sequence and not from knowledge about defined proteins. Bioinformatics tools were developed to select 50-150 amino acid antigen regions from coding parts of the genome and exclude regions with high sequence identity to other human proteins[4].

Approximately 20% of the published Triple A Polyclonals on the Human Protein Atlas are directed against target proteins with no “evidence at protein level” according to the Universal Protein Resource (UniProt) database. The Triple A Polyclonal catalogue is thus unique by containing so many antibodies towards proteins with unknown function and the Human Protein Atlas can assist in learning more about these proteins.

The Human Protein Atlas database can be searched for specific proteins using a string of queries including protein expression patterns, protein classes, validation of antibodies etc. Protein expression data is presented from 48 normal human tissue samples, 432 human cancer samples covering the 20 most common cancer types and from 59 different human cells and cell lines[5]. By narrowing search criteria, proteins with selective or cell type-specific expression patterns can be found. The three examples shown in Figure 1, 2 and 3 were found using the new and advanced search functions in the Human Protein Atlas database.

Annotated Protein Expression (APE) was introduced on HPA with the aim of presenting the combined results from several independent antibodies directed against the same protein target. Similar protein expression profiles from several Triple A Polyclonals towards non-overlapping regions of the same target strengthen the annotation of the unknown targets[1].

THE HUMAN PROTEIN ATLAS

The Human Protein Atlas is a public web portal managed by an academic project that aims to map the human proteome in a period of 10 years. More than 700 IHC, WB and IF images are presented for each antibody against human targets.

The antibodies developed and characterized within the Human Protein Atlas project are made available to the scientific community by Atlas Antibodies under the brand name Triple A Polyclonals.

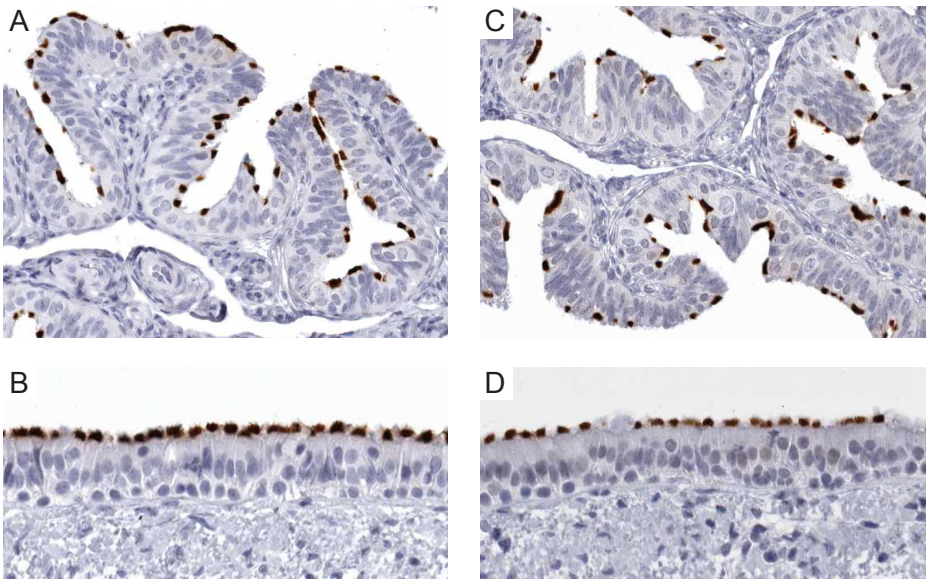

Figure 1.
Immunohistochemical staining using Anti-C1orf114 antibodies HPA027281 (A, B) and HPA027189 (C, D) in human fallopian tube tissue (A, C) and human nasopharyngeal tissue (B, D) shows strong immunoreactivity of ciliated epithelia. Recognition of target antigen is represented by brown color.

AAA POLYCLONALS
Designed by researchers, for researchers

Location Information Enhances Understanding

Data on where proteins are localized within tissues and cells provide important information as to what basic functions a protein may have as well as a possibility to map possible other interacting proteins.

In Figure 1, Triple A Polyclonals generated towards the putative protein encoded by the C1orf114 gene show a distinct and highly specific immunoreactivity in ciliated cells from different tissues. Four different antibodies towards non-overlapping regions of this alleged protein have been generated and all four show the same pattern of immunostaining.

The results from two of these (HPA027281 and HPA027189) are shown in fallopian tube and nasopharyngeal mucosa. The corresponding images display a localized expression of the C1orf114 protein in cilia of surface epithelial cells, indicating that this protein is involved in cilia function.

In Figure 2, two different Triple A Polyclonals generated towards a putative protein encoded by the C9orf11 gene, for which there is only evidence of existence on a transcript level, show a highly specific immunoreactivity in the innermost layer of maturing cells in the seminiferous ducts of testis. The localization of immunoreactivity is well in accordance with the acrosome and thus these results indicate that this protein is involved in sperm maturation and function.

In Figure 3, two different Triple A Polyclonals generated towards a putative protein encoded by the PDZK1 gene shows immunoreactivity in cells from normal kidney. A clear cytoplasmic expression pattern is displayed in cells of proximal tubules with a tendency to accentuated expression in the luminal brush border. Distal tubules show only a weak signal and cells of the glomerulus appear negative.

Triple A Polyclonals for Well Known Targets

In parallel to the analysis of proteins with unknown function, well characterized proteins are also used as targets for Triple A Polyclonal production. The reliable results achieved from these act as the optimal quality control for the whole Human Protein Atlas project.

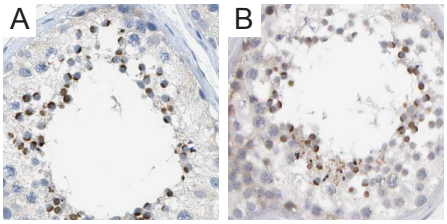

Figure 2.
Immunohistochemical staining using Anti-C9orf11 antibodies HPA015089 (A) and HPA015504 (B) in human testis tissue shows highly selective expression in acrosomes on spermatids. Recognition of target antigen is represented by brown color.

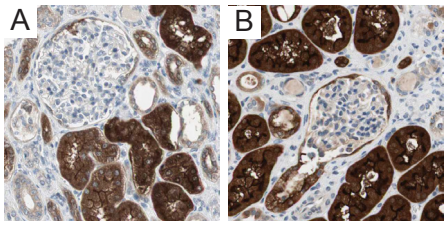

Figure 3
Immunohistochemical staining using Anti-PDZK1 antibodies HPA005755 (A) and HPA006155 (B) in human renal tissue shows strong specific expression in proximal tubes. Recognition of target antigen is represented by brown color.

References:

1) Uhlén M. et al. Towards a knowledge-based Human Protein Atlas. Nat Biotechnol. 2010 28(12):1248-50.

2) Berglund L. et al. A gene-centric human protein atlas for expression profiles based on antibodies. Molecular & Cellular Proteomics. 2008 7:2019-2027.

3) Asplund A. et al. Antibodies for profiling the human proteome - The Human Protein Atlas as a resource for cancer research. Proteomics. 2012 Jul;12(13):2067-77.

4) Berglund L. et al. A whole-genome bioinformatics approach to selection of antigens for systematic antibody generation. Proteomics 2008 8(14):2832-9.

5) Pontén F, Jirström K, Uhlén M. The Human Protein Atlas - a tool for pathology. J Pathology 2008 216(4):387-93.

Summary

• Triple A Polyclonals are developed from a genome-based strategy and 1/5 of the Triple A Polyclonals are directed against not yet characterized protein targets.

• Several independent Triple A Polyclonals against different regions of the same target protein verify the specific expression pattern of the target.

• Proteins with selective or cell type-specific expression patterns can be found using the search tools in the Human Protein Atlas database.

• More than 500 IHC images from normal and diseased human tissues are presented for each Triple A Polyclonal on the Human Protein Atlas portal (proteinatlas.org).

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