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Review Article

The Human Protein Atlas

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Introduction

Proteomics is the large-scale study of proteins, particularly their structures and functions.

The challenge for proteomics in the post-genome era is to utilize and understand the directions laid out by the human genome. Sequencing of the human genome has revealed approximately 20 500 protein encoding genes[1]. It is therefore pragmatic to consider a gene-centric proteomics approach in defining the complete human proteome for the human body[2].

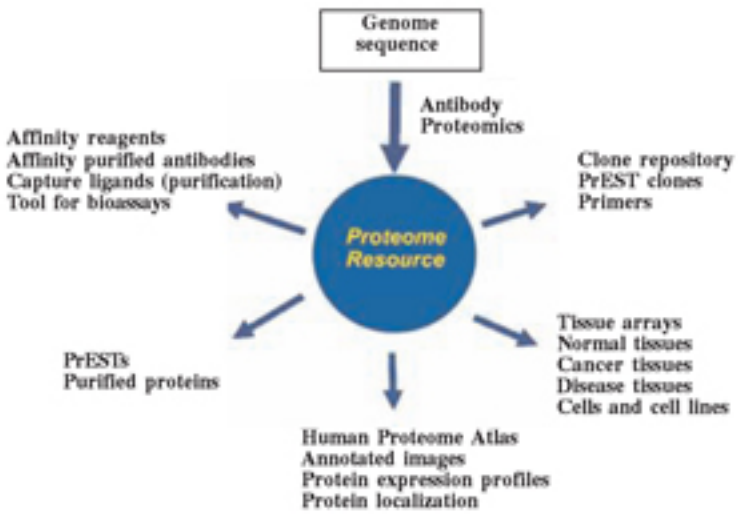

Fig. 1 The Challenge of Proteomics

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Antibody-Based Proteomics and The Human Protein Atlas Program

Antibody-based proteomics provides a powerful approach for the functional study of the human proteome involving the systematic generation of protein-specific affinity reagents. The Human Protein Atlas (HPA) program has been set up to allow for a systematic exploration of the human proteome using Antibody-Based Proteomics and is expected to deliver the first draft of the human proteome by the year 2014. This is accomplished by combining high-throughput generation of affinity-purified antibodies with protein profiling in a range of tissues and cells assembled in tissue microarrays. Confocal microscopy analysis using human cell lines is performed for more detailed, subcellular protein localization. The program hosts the Human Protein Atlas portal (www.proteinatlas.org) with expression profiles and images of human proteins in tissues and cells[3]. The new version 7 of the atlas, including more than 10 million images of immunohistochemically stained tissues and cells, is based on 13154 antibodies, representing 10118 genes comprising approximately 50% of the human genome.

The main sites for the HPA Program are located at the AlbaNova University center at the Royal Institute of Technology, Stockholm, Sweden, the Rudbeck Laboratory, Uppsala University, Uppsala, Sweden and Lab Surgpath, Mumbai, India. The main objective of the resource centre is to produce specific antibodies to human target proteins using a high-throughput production method involving the cloning and protein expression of Protein Epitope Signature Tags (PrESTs). After purification, the antibodies are used to study

expression profiles in cells and tissues and for functional analysis of the corresponding proteins in a wide range of platforms. The Stockholm site is responsible for generating high-quality antibodies and to perform the immunofluorescence analysis, the Uppsala site is responsible for large-scale protein profiling in tissues and cells using Immunohistochemistry. The objective of the Mumbai-HPA site to pursue evaluation and quality assured annotation of IHC-stained tissues for the Human Protein Atlas and for Biomarker discovery projects. The Mumbai-HPA site is also responsible for the initiation, supervision and collaboration for India-based research projects.

Tissue Microarrays and Immunohistochemistry

Immunohistochemistry or IHC refers to the process of detecting antigens (e.g., proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. The major limitations in immunohistochemistry analysis of tissues include the cumbersome nature of procedures, limited availability of diagnostic reagents, limited patient sample size and the high cost. The technique of tissue microarray was developed to address these issues. Tissue microarrays (also known as TMAs) consist of paraffin blocks in which several hundred up to 1000 separate tissue cores are assembled in array fashion on a single slide (Fig. 2). The TMA procedure facilitates Immunohistochemistry analysis by several different antibodies on sections from the same tissue block.

Adequate representation of tumor tissue needs careful consideration as only a limited amount of tissue is used in the TMA (usually 2-3 cores per tissue block, each core measuring between 0.6 mm to 2mm in diameter). Several studies have compared the use of sections from whole tissue blocks with different numbers and sizes of tissue cores in the TMA format. The use of two to four cores results in >95% accuracy[4]. The combination of immunohistochemistry and TMA technology is therefore an attractive high-throughput strategy, well suited for antibody-based proteomics[5].

Indian Surgical Pathology and The Human Protein Atlas

Indian surgical pathology contributes significantly to The HPA Program. Local surgical pathologists in Mumbai, India evaluate all tissues stained by immunohistochemistry on a "virtual" microscope (high resolution computer screen). More than eight million surgical pathology images have been annotated (evaluated) at the Mumbai site over the last four years.

Currently, approximately 200 antibodies are used on a routine basis in *diagnostic* surgical pathology employing the immunohistochemistry platform. The corresponding tissue protein profiles for these antibodies have been well characterized and form a useful tool in providing diagnoses in problematic cases. The HPA program, in comparison, will prepare a minimum of 20,000 *research* antibodies, one for every gene, and match the antibody expressions with the

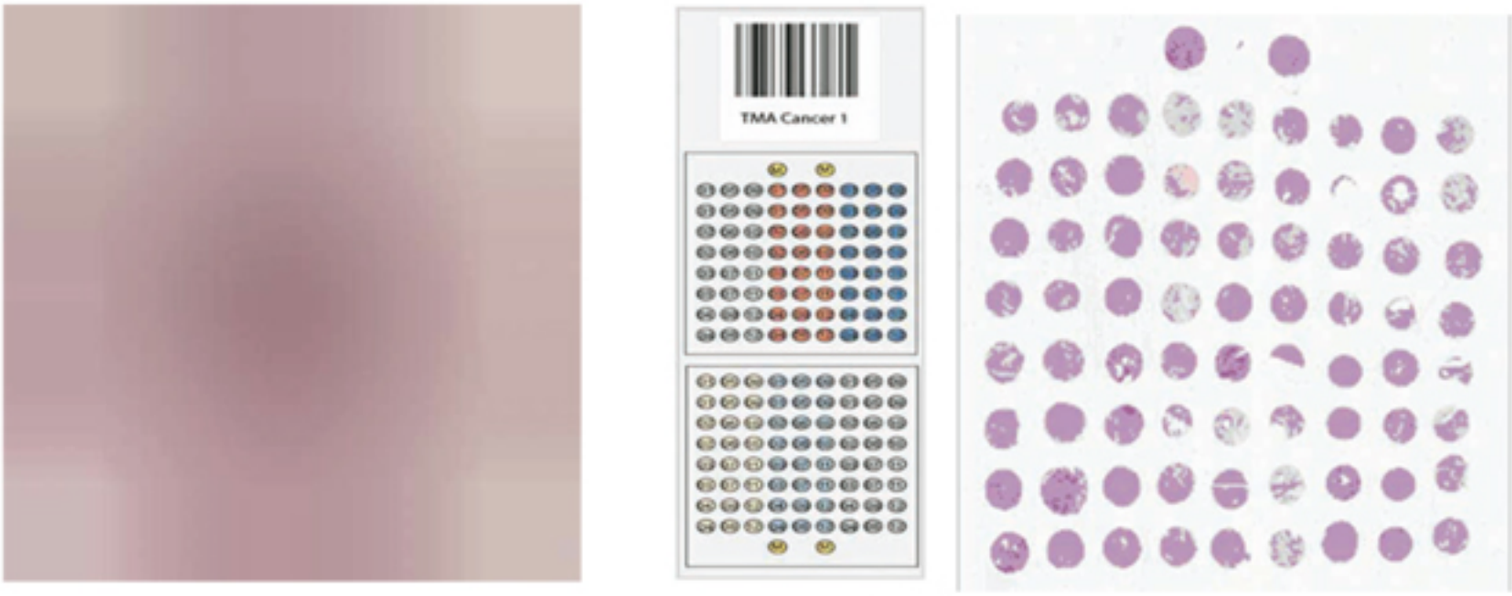


Fig. 2 Tissue Microarray (TMA)

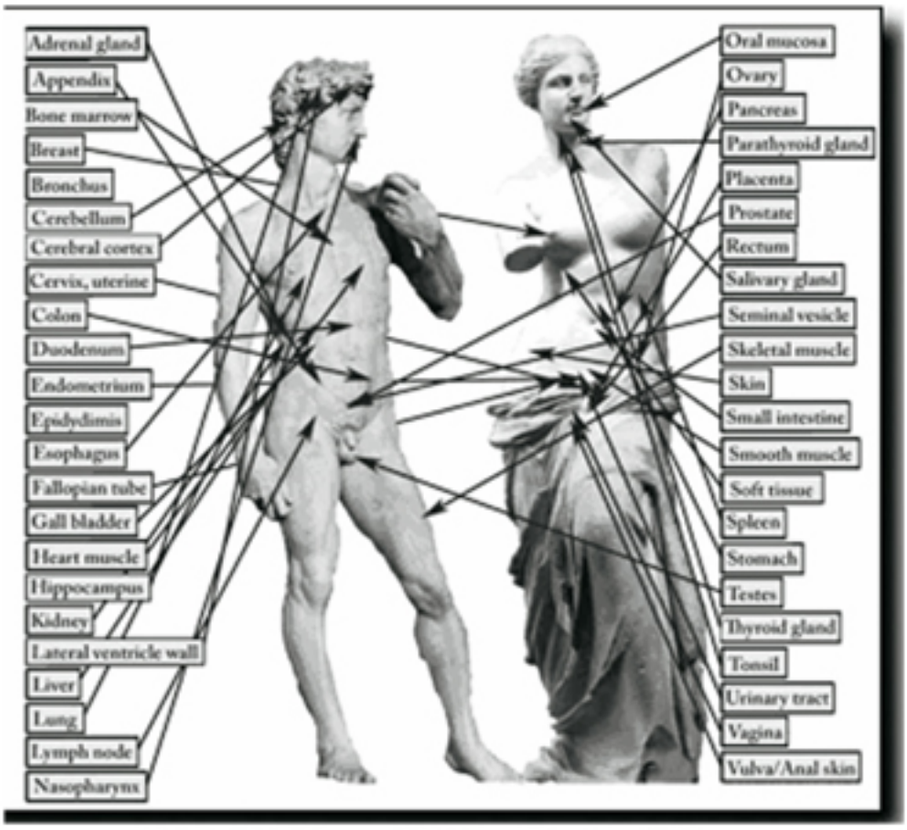


Figure 3 A. Normal Tissues Represented on The Human Protein Atlas

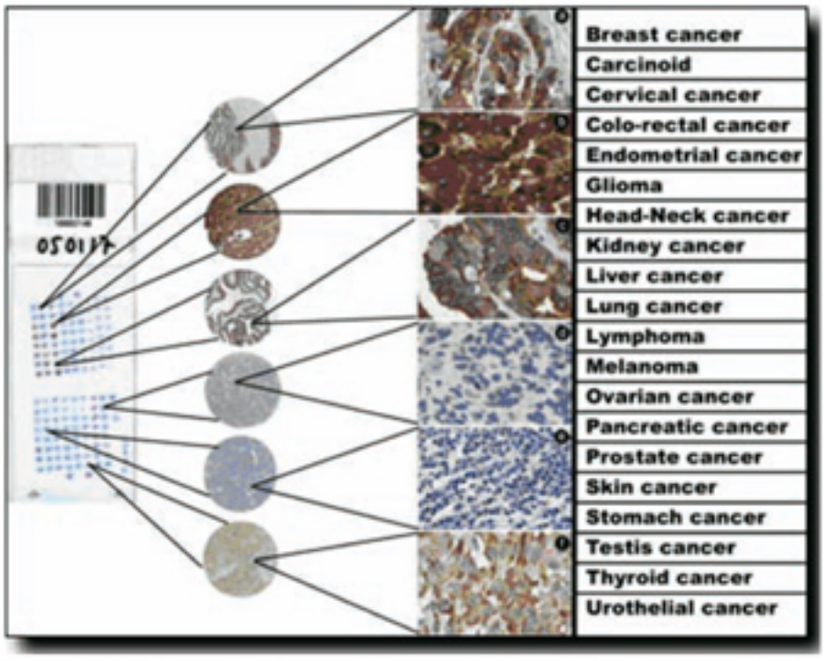
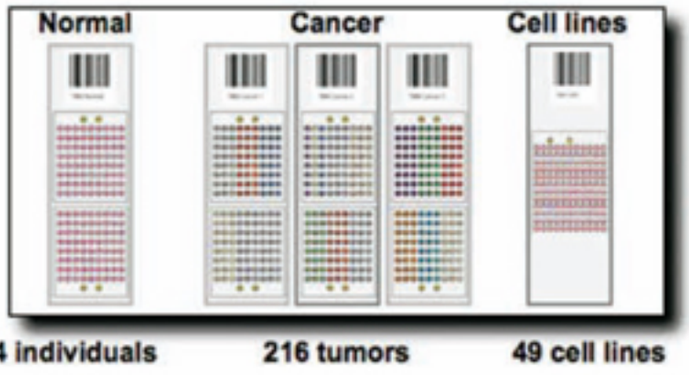


Figure 3 B. Tumor Tissues Represented on The Human Protein Atlas



Kampf et. al. "Antibody-based tissue profiling as a tool in clinical proteomics" Clin. Proteomics 1 285-299 (2005)

Andersson et. al. "Analysis of protein expression in cell microarrays: a tool for antibody-based proteomics" J Histochem Cytochem. 54(12):1413-23 (2006)

Figure 3 C. Tissue Microarrays Used in The Human Protein Atlas

proteins in various tissues by the year 2014. It is anticipated that this effort will help provide an insight into the pathogenesis of various disease states and develop diagnostic tools and therapies. Surgical pathologists are well-placed to evaluate the expression of various proteins as they harbour the unique knowledge and experience of tissue morphology.

The Human Protein Atlas Program evaluates each generated antibody on 708 different tissue and cell spots. The samples are derived from 46 different normal tissue types and 20 different types of cancer. For each antibody, the protein expression pattern in normal tissue is represented by triplicate samples and protein expression is annotated in 66 different normal cell types present in these tissues. For cancer tissues each tumor is represented in duplicate samples and protein expression is annotated in tumor cells. The generated tissue microarrays include samples from 46 different normal tissue types from 144 individuals and 20 different types of cancer from 216 patients.

Searching For Biomarkers, Epithelial Ovarian Cancer and The Role of RBM3

In addition to generating a map of protein expression patterns in a wide variety of human normal cells and tissues, the HPA portal allows for opportunities to identify proteins that show cell and tissue type-speciûc expression patterns, and to detect various types of cancer biomarkers. The effort is to provide for an improved stratification of disease and tumor groups classified on basic H&E pathology slides, into categories that share biologic characteristics and predictable therapeutic response[6].

The Indian Council of Medical Research (ICMR) Registry has reported the crude incidence of epithelial ovarian cancer (EOC) as 4.2 per 100,000 women, making it the fourth most common malignancy in Indian women[7]. EOC is the leading cause of death from gynaecological malignancy and fifth most common cause of cancer-related death in women in the United States. Most tumors present at an advanced stage and there is a lack of effective therapies for advanced refractory disease. Despite improvements in surgical techniques and the advent of more targeted therapeutic agents, five year survival rates for EOC are only 45%[8]. The statistics indicate an urgent need to improve on current understanding of the molecular mechanisms underlying EOC, so as to develop better early diagnostic and prognostic biomarkers. In addition, accurate predictive biomarkers are required to guide current treatment protocols, as well as to guide the development and application of new targeted therapies[9].

Standard treatment for advanced EOC involves surgical debulking followed by adjuvant chemotherapy with a combination of a platinum compound (cisplatin or carboplatin) and taxane[10]. After a primary response to cisplatin treatment, many patients with EOC develop resistance to the drug and relapse within a few years[11]. The molecular mechanisms underlying cisplatin resistance have been extensively studied and several mechanisms have been implicated[9].

Ehlen et al[9] utilized tissue microarrays to study RBM3 (a RNA binding protein) mRNA expression and RBM3 protein expression employing immunohistochemistry in two separate cohorts of 267 and 154 EOC cases. RBM3 protein expression was studied utilizing a monoclonal antibody that showed concordance with the polyclonal antibody for RBM3 developed within the HPA program. Both patient cohorts showed that high RBM3 mRNA expression and immunohistochemically identifiable RBM3 protein expression were associated with a significantly improved overall survival and increased sensitivity of EOC to cisplatin therapy. An association between nuclear expression of RBM3 in breast cancer (in two separate cohorts) and favorable clinicopathological parameters as well as a significantly improved survival irrespective of adjuvant treatment, especially in estrogen receptor (ER) positive tumors, has also been reported[12]. Ehlen et al hypothesized that RBM3 might enhance platinum-sensitivity of ovarian cancer cells *in vitro* and confirmed lower RBM3 protein levels in cisplatin-resistant ovarian cancer cell line A2780-Cp70 compared to their parental cisplatin-sensitive A2780 cells. The authors further demonstrated that silencing of RBM3 led to a decreased cisplatin response in ovarian cancer cells. The authors concluded that RBM3 is an independent marker of good prognosis and a predictor of response to platinum-based chemotherapy in EOC, most likely a combination of both. This view was particularly reinforced in the light of demonstrated good prognosis associated with RBM3 expression in breast cancer patients, where the vast majority of patients received no adjuvant systemic chemotherapy[12]. Further studies are required to evaluate the role of RBM3 in predicting response to other platinum-based agents, particularly in the setting of a prospective randomized control trial.

Research Possibilities in India

The Human Protein Atlas is a rich global resource that provides protein expression data on human normal and cancer cells. The current version displays protein profiles for 10118 genes, corresponding to approximately 50% of the human genome. This resource, with more than 10 million images, can be used for further analyses on disease and tissue-type specific patterns. Several unique reagents that were previously unavailable, are now generated through the HPA Program. These reagents provide a unique research toolbox that can be utilized in Indian studies to study various disease states involving the female reproductive tract. Proteomics research endeavours defining the protein expression patterns in periampullary carcinomas, malignant gliomas, congenital heart disease and skin tumors are currently underway in Mumbai. The role of Indian surgical pathologists who have evaluated the expression patterns within all these different tissues is an essential and desirable intellectual resource that can be utilized to further the cause of obstetrics and gynecological research in this country.

Acknowledgements

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