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Original Article

Antibody-Based Tissue Profiling As a Tool for Clinical Proteomics

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Abstract

Here, we show a strategy for high-throughput antibody-based tissue profiling with the aim to create an atlas of protein expression patterns in normal human tissues and cancer tissues representing the 20 most prevalent cancer types. A set of standardized tissue microarrays (TMAs) was produced to allow for rapid screening of a multitude of different cells and tissues using immunohistochemistry. Eight TMA blocks were produced containing 48 different normal human tissues in triplicate and cancer tissue from 216 individually different tumors in duplicate. Sections from these blocks were immunohistochemically stained using five commercial and five in-house generated antibodies. Digital images for annotation of expression profiles were generated using a semiautomated approach. Five hundred seventy-six images and annotation data corresponding to a total of 30 Gbytes of data were collected for each antibody. The data presented here suggest that antibody-based profiling of protein expression in tissues can be used as a valuable tool in clinical proteomics.

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Introduction

The completion of the human genome sequence and recent development of high-throughput technologies have set the stage for a clinical proteomics approach to analyze protein expression patterns on a genome-wide scale. The correlation between genotype and phenotype is poorly understood and presents a critical challenge to functionally explore mechanisms underlying normal development and disease. The phenotypical appearance as seen in a tissue section is evidently based on the pattern of expressed proteins within a cell as well as composition of the surrounding microenvironment. Microscopical evaluation of a tissue section taken from an excised tumor remains the golden standard for determining a diagnosis of cancer, although immunohistochemistry has provided additional support in cancer diagnostics *(1)*. In addition to determining expressed proteins, analysis of genomic DNA and transcribed genes add important information to the histological features detected in the microscope.

Immunohistochemistry provides a powerful tool for visualization of protein expression in tissues and cells. Immunohistochemistry has played an important role in clinical medicine as well as in basic research for defining different immunophenotypes present in tissues *(2)*. Major advancements in both basic knowledge and refinement of diagnostics have been made in the hematological field *(3,4)* and an increasingly important role in detection of different immunophenotypes is apparent in solid tumors *(5)*. Immunohistochemistry has, in addition, been important to study more general features, for example, proliferation and differentiation, and also been required to disclose otherwise invisible cell populations such as epidermal p53 clones *(6)*. One important objective for clinical proteomics is to generate a protein atlas displaying expression and localization patterns of proteins in all or most human tissues and organs *(7)*. Such a protein atlas would function as a general knowledge base with regard to the structural and temporal expression of the human proteins in various cells and tissues. Furthermore, such a database would also ultimately facilitate further studies to explore the biochemical function of the corresponding proteins.

To allow for such a proteomics strategy, it is important to develop high-throughput ways to generate and use specific antibodies that can be used as an immunohistochemical tool for analysis of biopsies from human patients. Agaton et al. showed recently that with bioinformatic selection of protein epitope signature tags (PrESTs), it is possible to generate highly specific antibodies in a high-throughput manner as exemplified by a pilot study using the genes of human chromosome 21 *(8)*. Protein fragments of the size 100–150 amino acid residues were expressed and purified from recombinant bacterial hosts and used to generate polyclonal antibodies. These antibodies were affinity purified using the PrEST as ligand *(9)*, and the purified antibodies were subsequently used for immunohistochemistry.

However, to allow for a comprehensive protein atlas covering most tissues and organs of the human body, a more systematic approach is needed for the immunohistochemistry to be able to analyze many tissues in parallel. Here, we describe the use of tissue microarray (TMA) for such high-throughput antibody-based tissue profiling. Immunohistochemistry combined with TMA technology presents an exceptional strategy for high-throughput analysis of protein expression in various tissues and cells *(7,10)*. The use of multiple biopsies or curettages in a single paraffin block has been used in surgical pathology procedures for several decades. Although several papers have

been published describing improvements of methodology and usefulness in immunohistochemistry *(11–13)*, multitissue blocks did not become a powerful tool in research prior to important standardization described by Kononen and coworkers *(14)*.

Here, we show that high-throughput analysis of protein expression can be performed with a standard set of TMAs representing both normal and cancer tissues. The TMA technology used in this study provides an automated array-based high-throughput technique in which as many as 1000 paraffin-embedded tissue samples can be brought into one paraffin block in an array format. The diameter of the tissue sample cores is small, here 1.0-mm diameter, to prevent loss of valuable and unique tissues. Using this strategy, we were able to generate 576 standard digital immunohistological images for every antibody studied. This approach is possibly to scale-up for whole proteome efforts in which a large number of protein profiles are generated by analyzing these tissue arrays.

Materials and Methods

Antibodies

Five commercially available antibodies, with well-known staining properties, used in routine diagnostic immunohistochemistry were selected (Table 1). These antibodies were chosen to represent different staining patterns in human tissues including various subcellular compartments, that is, nucleic (Cyclin A), membranous (HER-2 and CD 34), and cytoplasmic (Keratin 14, GFAP).

The generation of affinity-purified antibodies was performed essentially as previously described *(8)*. In brief, PrESTs corresponding to a size of 100–150 residues were defined using bioinformatic tools with the human genome sequence as template (Ensembl database, http://www.ensembl.org/). Following RT-PCR and cloning, recombinant proteins were produced and purified for immunization. Polyclonal antisera were subsequently affinity purified *(9)* and tested for specificity and sensitivity using PrEST arrays prior to immunohistochemical staining. In the present study, five such PrEST antibodies were used (Table 1). The PrEST antibodies included a few antibodies recognizing proteins for which there has been previously described expression patterns, for example, K230002 recognizing a zinc finger protein with probable nuclear localization; K230003 recognizing a G protein coupled receptor-associated sorting protein (GASP) with possible membranous localization; K230008 recognizing polyadenylate binding protein 5 with possible cytoplasmic localization; and K230009 recognizing dyskerin with expected nucleolar localization. For the antibody K230011, there was no description.

Tissue Microarrays

Tissue and Tissue Processing

In total, 576 paraffin cores containing human tissues were analyzed for each antibody. All tissues used as donor blocks for TMA production were selected from the archives at the Department of Pathology, University Hospital, Uppsala, in agreement with approval from the local ethical committee. Corresponding tissue sections were examined to determine diagnosis and to select representative areas in donor blocks. Normal tissue was defined as microscopically normal (non-neoplastic) and was most often selected from specimens collected from the vicinity of surgically removed tumors. Cancer tissue was reviewed for diagnosis and classification. All tissues were formalin fixated, paraffin embedded, and sectioned for diagnostic purposes.

TMA Production

The TMA production was performed essentially as earlier described *(14,15)*. Briefly, a hole was made in the recipient TMA block. A

Table 1
Antibodies Used in the Present Study

Antibody	Gene ID	Gene name	Gene description	Source	Species	Antigen retrieval	Dilution
HPRK 230002	ENSG0000 0125352	ZNF183[a]	Zinc finger protein 183	In-house	R	TE	1:200
HPRK 230003	ENSG0000 0125961	NM_014710[b]	G protein-coupled receptor-associated sorting protein	In-house	R	TE	1:100
HPRK 230008	ENSG0000 0174740	PABPC5[a]	Polyadenylate-binding protein 5	In-house	R	TE	1:250
HPRK 230009	ENSG0000 0130826	DKC1[a]	Dyskerin (nucleolar protein NAP57)	In-house	R	TE	1:200
HPRK 230011	ENSG0000 0157502	NM_152423[b]	No description	In-house	R	TE	1:100
CD34	ENSG0000 0174059	CD34[a]	Hematopoietic progenitor cell antigen CD34 precursor	DakoCytomation	M	TE	1:100
Cyclin A	ENSG0000 0113558	SKP1A[a]	S-phase kinase-associated protein 1A	Novocastra[c]	M	TE	1:200
GFAP	ENSG0000 0131095	GFAP[a]	Glial fibrillary acidic protein, astrocyte	DakoCytomation	R	Protease	1:2000
HER-2	ENSG0000 0141736	HER-2/ ERBB-2[a]	Receptor protein–tyrosine kinase ERBB-2 precursor	DakoCytomation	R	TE	1:1500
Keratin 14	ENSG0000 0186847	KRT 14[a]	Keratin, type 1 cytoskeletal 14	Lab Vision/ Neomarkers[d]	M	TE	1:800

Corresponding Ensembl ID, protein description, source of antibody, and immunostaining parameters are given.
[a]HUGO ID.
[b]RefSeq ID.
[c]Newcastle upon Tyne, UK.
[d]Fremont, CA, USA.
R, rabbit polyclonal ab; M, mouse monoclonal ab; TE, Tris-EDTA, pH 9.0.

Table 2
All Utilized Normal Tissues

Normal tissue	
Adrenal gland	Parathyroid gland
Appendix	Placenta
Breast	Postmenopausal endometrium
Bronchus	Premenopausal endometrium
Bone marrow	Prostate
Cerebellum	Rectum
Cerebral cortex	Salivary gland
Duodenum	Seminal vesicle
Epididymis	Skin
Esophagus	Small intestine
Fallopian tube	Smooth muscle
Gall bladder	Soft tissue 1
Heart muscle	Soft tissue 2
Hippocampus	Spleen
Kidney	Striated muscle
Large intestine	Testes
Lateral ventricle	Thyroid gland
Lower stomach	Tonsil
Liver	Upper respiratory tract
Lung	Upper stomach
Lymph node	Urinary bladder
Oral mucosa	Uterine cervix
Ovary	Vagina
Pancreas	Vulval-anal skin

Triplicate samples were collected from each normal tissue and used as templates for the two normal tissue microarray blocks.

Table 3
All Cancer Tissues Including the Number of Individual Tumors Used

Cancer type	Individual tumors
Breast cancer	12
Cervical cancer	12
Colorectal cancer	12
Endometrial cancer	12
Head-neck cancer	4
Kidney cancer	12
Liver cancer	12
Lung cancer	12
Malignant carcinoid	4
Malignant glioma	12
Malignant lymphoma	12
Malignant melanoma	12
Ovarian cancer	12
Pancreatic cancer	12
Prostate cancer	12
Testes cancer	12
Thyroid cancer	4
Skin cancer	12
Stomach cancer	12
Urothelial cancer	12

Duplicate samples were collected from each individual tumor and used as templates for the six cancer tissue microarrays.

cylindrical core tissue sample from the donor block was acquired and deposited in the recipient TMA block. This was repeated in an automated tissue arrayer from Beecher Instrument (ATA-27, Beecher Instruments, Sun Prairie, CA) until a complete TMA design was produced. TMA recipient blocks were baked at 42°C for 2 h prior to sectioning.

TMA Design

The design of TMAs was emphasized on obtaining samples from a large range of representative normal tissues. Selection was limited to include only tissues from which enough material could be taken to allow for a large number of sample biopsies to be sent to pathology for diagnosis. The other main focus was to include representative cancer tissues. We chose to include 20 of the most common cancer types that affect humans. For 17 of these cancer types, we included 12 individually different tumors. In total, eight different designs of TMA blocks containing 72 cores each of tissue with 1-mm diameter were produced. The spacing between each core center is 2 mm in both *x*- and *y*-axis. Two of these TMAs included normal tissues (Table 2) and the remaining 6 TMAs contained cancer tissue from 20 different types of cancer (Table 3). From 17 of these cancer types, 12 individually different tumors were sampled and from the remaining 3 tumor types, 4 individually different

tumors were sampled. For normal tissues, triplicate samples from different individuals were used and from cancer tissues, duplicates from the same tumor were used (schematically shown in Fig. 1). Four-micrometer thick sections were cut and two such sections were placed on each glass slide.

Immunohistochemistry

Five commercially available antibodies and five PrEST antibodies were selected for this pilot study (Table 1). For the set of commercial antibodies, manufacturers' recommendations were used for antigen retrieval and concentration of primary antibody. For the PrEST antibodies, a test strategy was used to determine methods of antigen retrieval and antibody concentration. Slides containing various tissues were used to test different modes of retrieval and concentration in order to obtain a final protocol for immunohistochemical staining. The immunohistochemical staining was performed as previously described *(16)* using a system for automated immunohistochemistry (Autostainer, DakoCytomation, Glostrup, Denmark). To test for specific binding, we mixed primary antibodies with a dilution series of respective PrEST antigen prior to incubation of tissue sections. The adsorption tests resulted in quenching of immunoreactivity. When primary antibodies were adsorbed with 25 µg/mL, there was no detectable specific immunoreactivity.

Digital Imaging of the Tissue Cores

All immunohistochemically stained sections from the eight different TMAs were scanned using two automated slide-scanning systems, the ACIS II (ChromaVision, San Juan Capistrano, CA) and the ScanScope T2 (Aperion Technologies, Vista, CA). For each antibody, 576 digital images were generated to represent the total content of the eight TMAs. Scanning was performed at ×20 magnification. Digital images were separated and extracted as individual TIFF files for storage of original data. The size of each TIFF image is approx 50 Mbytes, depending on how close to the core the image separation has occurred, that is, the amount of white space around the core. Each TMA will generate 3.6 Gbytes (72 × 50) of data; that is, each antibody staining (eight TMAs) will generate approx 30 Gbytes of images excluding the database with annotation data. The high-resolution TIFF images are stored on digital tapes but the JPEG images and the database are on-line, enabling instant access.

Scoring of Protein Expression

The annotation software was developed to allow for a basic and rapid evaluation of immunoreactivity in a broad spectrum of different tissues and cells. The annotation tool software was developed to run on any standard desktop PC (Windows and Macintosh operating systems) using an ordinary Web browser interface. To handle the images in a Web-based annotation system, we compressed the individual images from TIFF format into JPEG format. Parameters that were annotated included overall staining, congruity in staining between triplicate/duplicate samples, and reliability of immunohistochemical staining, as well as staining intensity, fraction of immunoreactive cells, pattern, and localization of immunoreactivity (nuclear, cytoplasmic, or cell membranous). A text box was also included for comments.

Results

Design of TMAs Representing Normal Tissues

Two TMA blocks with 72 cores each were designed containing normal tissues representing a broad spectrum of human organs and tissues (Table 2). In all, 48 different tissues were selected to represent the majority of organs and tissues in the human body. Selection of normal tissues was further based

Fig. 1. Figure illustrating tissue microarray (TMA) design. The two normal TMAs containing 144 cores placed on one glass slide is shown (left). A schematic example of two cancer TMAs containing 144 cores from six different types of cancer is shown (right).

on the availability in pathology archives in order to secure a constant design for screening on a genome-wide scale. Consequently, certain specialized tissues, for example, spinal chord, ear, and eye tissues, were omitted. To avoid loss of data from individual differences in expression patterns or technical failures, we used triplicate samples. These samples were selected from three different individuals and, when applicable, both sexes were represented.

Design of TMAs Representing Cancerous Tissues

Six TMA blocks with 72 cores each were designed containing cancerous tissues representing the most common forms of cancers. As heterogeneity is a hallmark of cancer, we chose to include several tumors from each tumor type in order to accomplish an acceptable representation of tumor type. From 17 tumor types, we selected tumors from 12 different patients and from 3 tumor types, we selected 4 individually different tumors (Table 3). Because we had a fairly large number of tumors in each category, a duplicate sample from each tumor was found sufficient to avoid loss of data. One example of two cancer TMA designs on a slide is shown in Fig. 2.

Production of the TMAs

Altogether, eight different types of TMA blocks were produced with two TMAs corresponding to normal tissues and six TMAs corresponding to the cancerous tissues. Each TMA block allowed for cutting of approx 250 sections with a thickness of 4 μm. In total, 5760 cores were sectioned and immunohistochemically stained. From the expected 5760 cores stained and scanned, 231 cores were missing (4%). Four hundred of the cores were not representative, that is, did not include selected normal tissue type or failed to include selected cancer cells. The remaining 5129 stained spots were used as templates for evaluation and annotation. After exclusion of missing images and nonrepresentative images, 89% of theoretically possible tissues were available for analysis.

Analysis of the TMAs With Commercial Antibodies

Five commercial antibodies were selected to represent different expression patterns found in tissues, that is, Cyclin A (proliferation in cancer); HER-2 (HER-2 receptor positive breast cancer); blood vessels (CD 34); brain tissue (GFAP); and basal cells in squamous epithelia (Keratin 14). The selection of antibodies was furthermore based on different subcellular compartments, including nucleus (Cyclin A), cell membrane (HER-2 and CD 34), and cytoplasm (GFAP and Keratin 14). Immunohistochemical staining of TMAs using the selected commercial antibodies produced protein expression patterns in accordance with earlier experience and published data. In general, immunohistochemical staining produced patterns with low background and high specificity. Some examples are shown in Fig. 3. Confirmation of expected staining pattern included the following: (1) Clear staining of vascular structures was evident using the anti-CD34 antibody; (2) cyclin A immunoreactivity coincided with what was expected for a marker of a specific cell cycle phase in cancer cells as well as certain normal proliferating tissues; (3) the HER-2 antibody showed a clear membranous staining pattern in a subset of breast and other adenocarcinomas; (4) GFAP staining was exclusively found in brain tissue and malignant gliomas; and (5) Keratin 14 staining showed cytoplasmic immunoreactivity in epithelial structures known to express Keratin 14.

Analysis of the TMAs With PrEST Antibodies

Five PrEST-specific antibodies were also analyzed in the same manner to show that an antibody-based proteomics approach can be

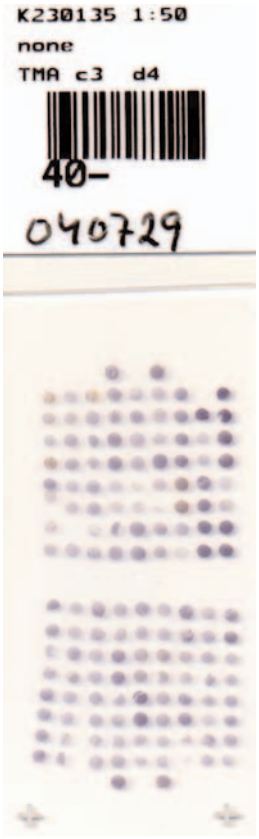

Fig. 2. Image showing immunohistochemically stained tissue microarrays (TMAs). The figure shows an immunostained glass slide containing tissue sections from two cancer TMAs stained with an in-house generated antibody.

used to generate the antibodies used to analyze the tissues in the produced TMAs. Examples of the immunohistochemical pattern of the PrEST antibodies are shown in Fig. 4; expression patterns are shown in normal tissues (Fig. 4A) and cancer tissues (Fig. 4B). PrEST antibody K230002, generated toward zinc finger protein 183, displayed a prevalent nuclear immunoreactivity in normal as well as cancerous cells, with enhanced cytoplasmic immunoreactivity in Leydig cells of the testes and cells in proximal tubules of normal kidney. The K230003 antibody generated toward GASP showed a distinctly different expression pattern, with membranous staining in placenta, immunoreactivity in luminal lining of tubules in the kidney, and a clear vascular pattern in the spleen. Colorectal adenocarcinomas also showed a widespread positivity along luminal surfaces. The antibody generated toward polyadenylate binding protein 5, K230008, showed a strong positive staining in maturing germinal cells of the testes and cells in the cerebellum, including Purkinje cells, whereas cancer tissues only showed a weak cytoplasmic staining pattern. The PrEST antibody K230009 generated toward dyskerin (nucleolar protein NAP57) showed a clear immunoreactivity in the nucleoli of most cells, especially evident in cells such as keratinocytes, Purkinje cells, and several cancer cells. For the K230011 antibody, generated toward a protein for which there is no description, a positive staining pattern was found in immature germinal cells of the testes, molecular layer in cerebellum, and outer membrane of proximal tubules of the kidney. In cases where bioinformatics data on gene/protein expression were available, the outcome of the immunohistochemical staining using PrEST antibodies on TMAs was compatible with specific binding of the respective antibody. Adsorption with corresponding PrEST protein prior to incubation of primary antibody abolished specific immunoreactivity in all cases.

Digital Imaging of the Tissue Cores

Individual images of all 576 (72 cores × eight TMAs) immunostained cores from the TMAs were extracted and stored in a database. One slide containing two TMAs (two TMAs × 72 cores) was scanned and stored as an original large TIFF file representing 144 cores. This image was divided using image-extraction software into 144 separate TIFF images, each representing one individual core. JPEG images were subsequently generated in several resolutions to enable Web-based viewing and annotation. A brief text annotation was made by a pathologist

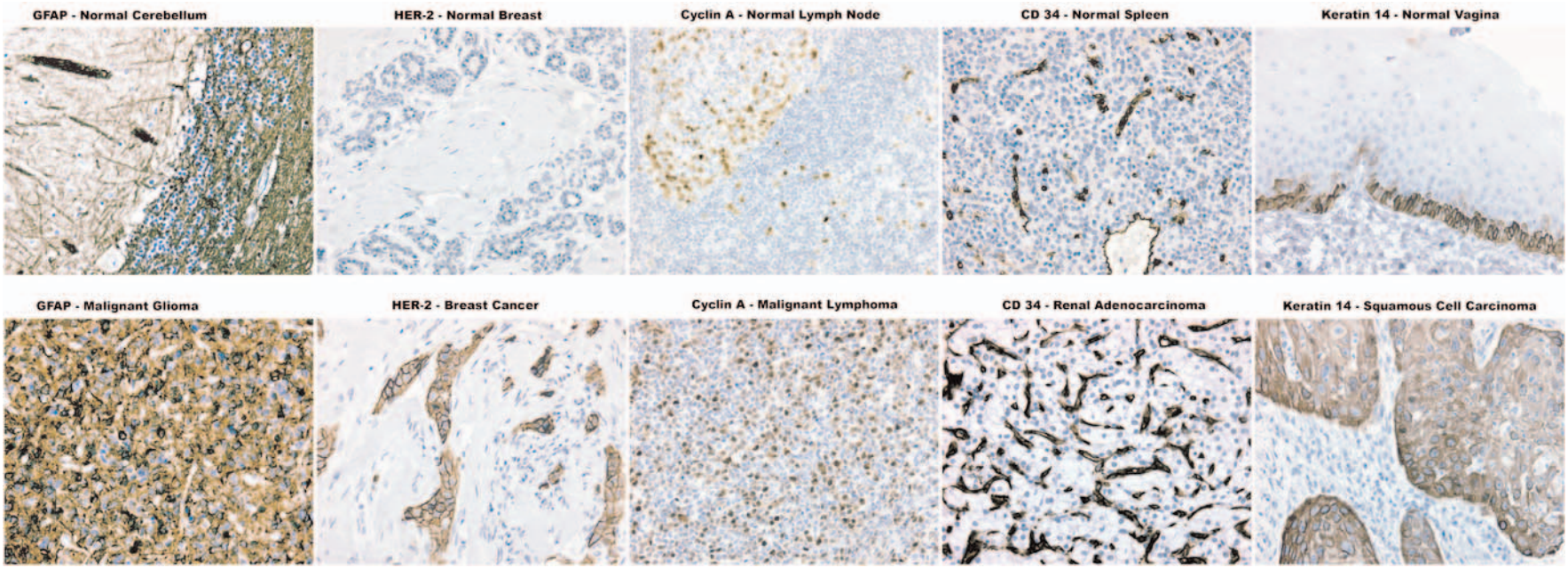

Fig. 3. Image showing examples of immunostained tissues from tissue microarrays (TMAs) stained with the commercial antibodies. Top row shows examples of normal tissues and bottom row shows examples of cancer tissues stained with the various antibodies. Antibodies used from left to right were GFAP (normal brain tissue and malignant glioma), HER-2 (normal breast tissue and breast carcinoma), Cyclin A (normal lymph node and malignant lymphoma), CD 34 (normal spleen and renal adenocarcinoma), and Keratin 14 (normal vaginal mucosa and cutaneous squamous cell carcinoma). All images derived from slides scanned at $\times$20 magnification.

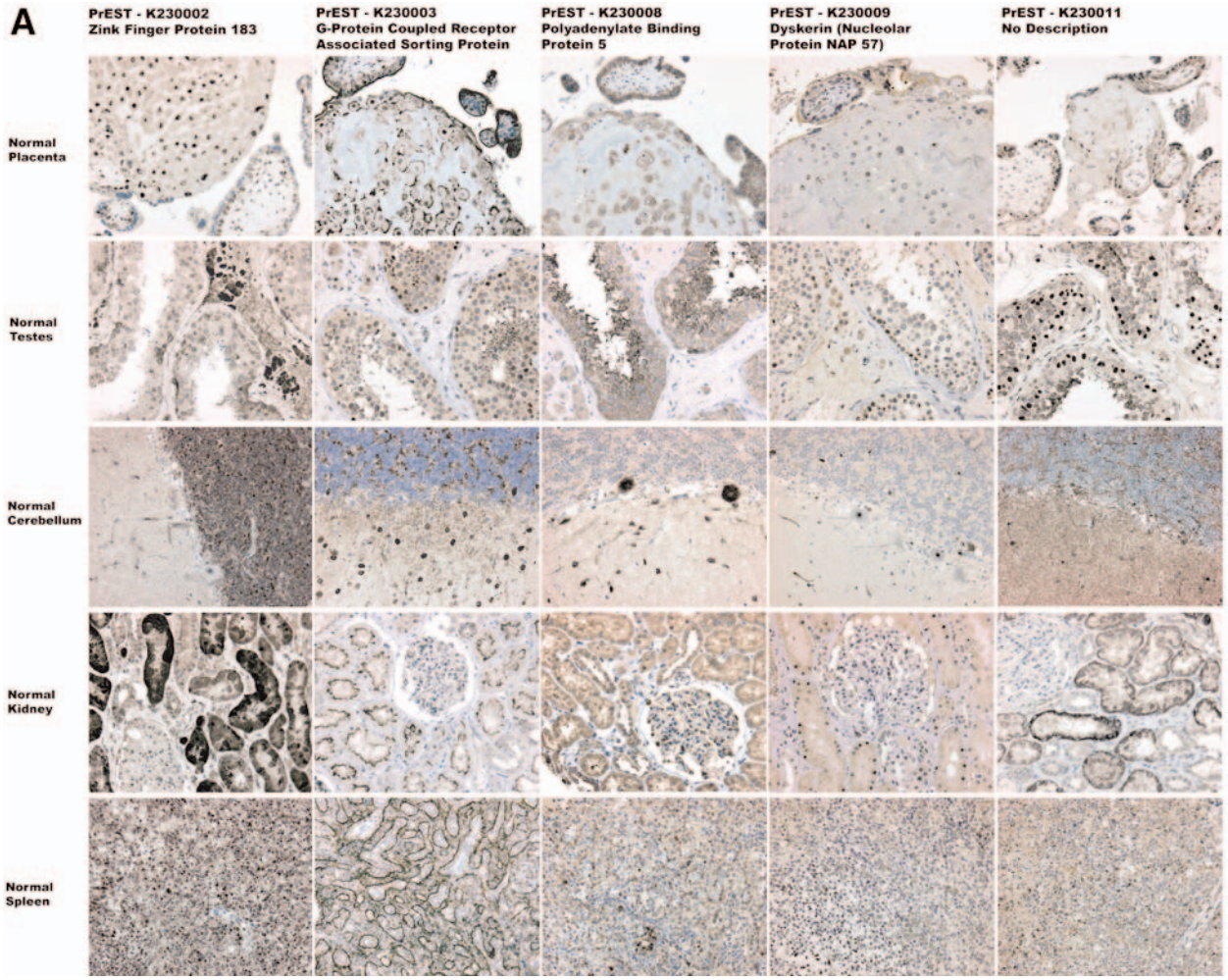

Fig. 4. **(A)** Examples of normal tissues stained with the in-house-generated protein epitope signature tag (PrEST) antibodies. Images shown from top to bottom include normal tissues from placenta, testes, cerebellum, kidney, and spleen. Immunoreactivity for HPRK230002, HPRK230003, HPRK230008, HPRK230009, and HPRK230011 antibodies are shown from left to right. Note the low background staining and clear differences in staining patterns in the different tissues. **(B)** Examples of cancer tissues stained with the same antibodies as in **A**. Cancer tissues shown include, from top to bottom: prostate carcinoma, colorectal carcinoma, and two different cases of malignant glioma. Parallel sections from the same tissue microarray blocks were used to facilitate a comparison of the results from the different antibodies used.

and the following parameters were scored: overall staining (negative or positive); congruity (congruent or conflict); reliability (distinct, probable, or uncertain); and, for tissue-specific cells, intensity (strong, moderate, weak, or negative); fraction of positive cells (>75%, 25–75%, <25% or rare cells); staining pattern (homogenous or heterogeneous); and localization (nuclear, cytoplasmic, membranous, and/or extracellular). All data were saved together with the corresponding images in a database.

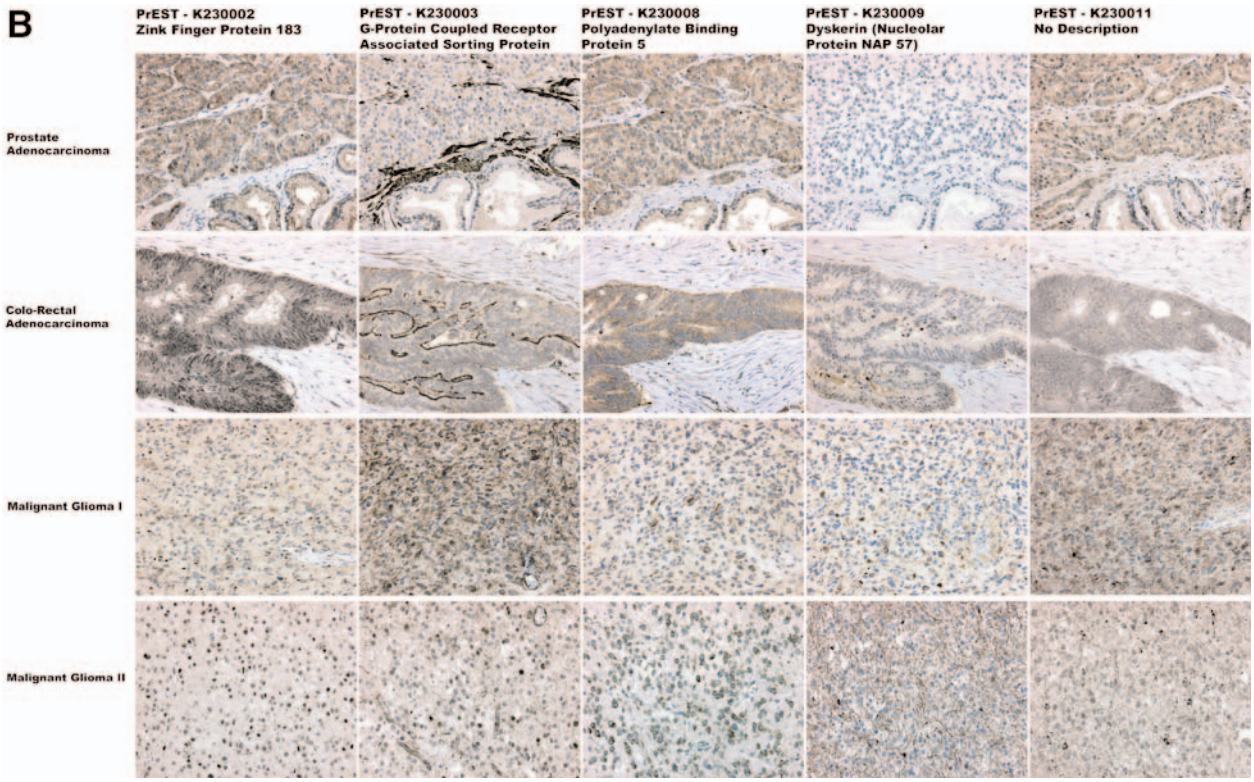

Fig. 4. (*continued*)

Discussion

In this paper, we describe a strategy based on antibody proteomics including TMAs to analyze protein expression patterns in a large number of normal and neoplastic human tissues. The strategy has been to develop a scalable system that can be used on a whole proteome scale using tens of thousands of specific antibodies. Each antibody will generate 576 high-resolution images corresponding to normal and cancer tissues. In this manner, a protein atlas for tissue expression and localization will be built up for each protein with an available specific antibody. Here, we show that both commercial antibodies and affinity-purified polyclonal antibodies can be used to generate the data for the protein atlas.

The success for immunohistochemistry in a clinical proteomics setting relies on the quality of antibodies and tissues used *(7,17)*. We have chosen commercially available polyclonal and monoclonal antibodies with well-known expected staining patterns as examples, as well as antibodies generated toward unique recombinant protein fragments based on the human genome sequence *(8)*. Commercial antibodies were chosen to represent different subcellular localization patterns and used as control for immunostaining. To optimize protocols for immunostaining with PrEST antibodies, a series of tests including different modes of antigen retrieval *(18)* and different concentrations of primary antibody was performed prior to staining of the eight TMAs. In cases where expression data were available, staining patterns with PrEST antibodies were consistent with a specific immunoreactivity for the given protein. For proteins with unknown expression patterns, there

is no given true result; however, patterns of immunoreactivity were evaluated by certified pathologists, and adsorption with PrEST antigen prior to immunostaining quenched the pattern of immunoreactivity as expected for a specific immunostaining.

Antibody-based tissue profiling allows a streamlined approach for generating expression data, both for normal and disease tissue. Our strategy for selection of tissues included eight different TMA designs. The chosen strategy, which includes 48 different samples of normal human tissues in triplicate and duplicate samples from 216 individually different malignant tumors representing the 20 most common forms of human cancer, provides a first attempt to screen for different protein expression patterns. As exemplified here for cancer, it is possible to generate data on many different individual patients to evaluate heterogeneity of tissue profiles. It is of course possible to also include the analysis of other clinically important diseases as long as clinical material can be obtained in sufficient amounts. Degenerative diseases, cardiovascular and metabolic diseases, infectious diseases, and neurological disorders are examples of disease that are suitable for antibody-based tissue profiling as described here. Consequently, it will also be important to extend the analysis of protein expression in different stages of development and in other species. The fact that paraffin-embedded biosamples can be used allows the inclusion of archive material.

Scoring and annotation of protein expression was performed by a certified pathologist to ensure a high quality of microscopical analysis and evaluation of immunoreactivity *(19)*. Complex tissue includes the existence of several different cell types acting in concert to execute normal organ function. A section from a normal tissue will thus contain cells with distinct and different phenotypes, including differences in immunoreactivity for various antibodies. In certain tissues, only a minority of present cells determines a specific function of a defined tissue, for example, islet cells in pancreatic tissue or glomerulus cells in kidney tissue. For a meaningful assessment of protein expression profiles, it is thus not possible to merely measure intensity and extent of immunoreactivity for a crude tissue core as such. To acquire a sufficient resolution for analysis, we have included the major cell types that comprise a defined normal tissue, for example, for cerebellum Purkinje cells, cells of molecular layer and cells of granular layer were analyzed separately and for uterus endometrial gland cells, cytogenic stroma cells and myometrial smooth muscle cells were analyzed as different compartments. The level of detail for a sufficient analysis can be debated. For high-throughput tissue profiling, time for annotation is an important and limiting factor. However, a database containing high-resolution images enables more in-depth analysis as well as use of potential automated image analysis tools in the future *(20)*. Because normal and cancer specimens were not patient matched, it cannot be ruled out that differences in staining in part are from differences in procurement and fixation rather than from true biological differences. To compensate for this to some extent, we included triplicate samples from 3 different individuals as normal tissues and 12 individually different tumors for representation of different forms of cancer. It is unlikely that differences from treatment of tissues would cause systematic errors.

To furthermore study protein expression in defined cells, we have designed a TMA from in vitro cultured cells *(21)* and patient cell samples (Stromberg, S. et al., unpublished data). Such a cell-TMA facilitates a comparative analysis of transcription and protein expression using immunohistochemistry combined with image analysis and cDNA-array technologies. In addition, cell-TMAs provide enhanced

possibilities to study hematological malignancies for which there are readily available cell aspirate samples as well as a multitude of established cell lines for which data on genetic background, transcription profiles, and protein expression levels have been published. To a lesser degree, cell-TMAs can also be used as model systems for solid tumors and provide a useful validation for selected antibodies.

Antibody-based tissue profiling is an important complement to transcript profiling. As a large amount of data is generated through the use of microarray technologies, there is an increasing demand to validate and compare transcription profiles with protein expression profiles in various normal as well as diseased tissues. Subclassification of different cancer types by gene expression analysis requires optimal experimental design as well as sophisticated management of data *(22)*. Several examples exist in which differences in transcription profiles have suggested that cancer types, as defined through conventional histology, include several subtypes with differences in behavior, prognosis, and sensitivity to therapy, for example, breast cancer *(23,24)*. The presented strategy permits high-throughput tissue profiling of specific antibodies on a genome-wide scale and would provide an excellent basis for validation of up- and downregulated genes. Furthermore, one could envision the development of specific antibody panels as tools for diagnostic and prognostic information in given types of cancer. Immunohistochemistry allows for an enhanced understanding of morphology as it adds important information based on specific immunoreactivity in defined cell populations. Today in clinical medicine, analysis of immunohistochemical profiles as well as mutation analysis and levels of transcription can add important information regarding prognosis and choice of treatment for certain forms of cancer.

One of the advantages with the TMA approach for generating tissue profiles is the ease of automation for scanning of the immunohistochemical cores using various commercial software and hardware. Several different technologies for rapid scanning of glass slides containing a tissue section have been developed *(25)*. One major advantage of these techniques is the generation of digital data available for analysis. In a first pass, digital images can be viewed and scored using conventional Web-based tools and thus primary data will be easily accessible. In order to handle large file sizes using a Web-based interface, our image files were compressed from TIFF to JPEG files. Each image representing a 1-mm core could be reduced from approx 50 Mbytes to 1 Mbyte without detectable impairment of image quality on an ordinary screen. The reduced file sizes allows for rapid transition between images while performing annotation. The compressed images also serve as a basis for publishing primary data on the Web. Although challenging, it is not impossible to handle these large amounts of data.

In summary, we have shown that an antibody-based proteomics strategy can be used to systematically explore clinical tissues using a standardized set of TMAs. Using eight TMA blocks with altogether 576 individual tissue cores, it was possible to generate tissue profiles including expression levels and localization data, which are stored as digital images suitable for Web-based publishing. With the approach described here, it should be possible to scale up to generate tissue profiles for tens of thousands of antibodies. Thus, a comprehensive protein atlas for a large part of the human proteome is possible if specific antibodies can be generated within the framework of various international efforts.

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