Histol Histopathol (2004) 19: 325-335
DOI: 10.14670/HH-19.325

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Post-genomic applications of tissue microarrays: basic research, prognostic oncology, clinical genomics and drug discovery

A. Mobasheri[1], R. Airley[2], C.S. Foster[3], G. Schulze-Tanzil[4] and M. Shakibaei[4]

[1]Molecular Pathogenesis and Connective Tissue Research Groups, Department of Veterinary Preclinical Sciences, Faculty of Veterinary Science, University of Liverpool, Liverpool United Kingdom, [2]School of Pharmacy and Chemistry, Liverpool John Moores University, Liverpool, United Kingdom, [3]Department of Cellular and Molecular Pathology, Faculty of Medicine, University of Liverpool, Liverpool, United Kingdom and [4]Institute of Anatomy, Charité Medicine University Berlin, Campus Benjamin Franklin, Berlin, Germany

Summary. Tissue microarrays (TMAs) are an ordered array of tissue cores on a glass slide. They permit immunohistochemical analysis of numerous tissue sections under identical experimental conditions. The arrays can contain samples of every organ in the human body, or a wide variety of common tumors and obscure clinical cases alongside normal controls. The arrays can also contain pellets of cultured tumor cell lines. These arrays may be used like any histological section for immunohistochemistry and *in situ* hybridization to detect protein and gene expression. This new technology will allow investigators to analyze numerous biomarkers over essentially identical samples, develop novel prognostic markers and validate potential drug targets. The ability to combine TMA technology with DNA microarrays and proteomics makes it a very attractive tool for analysis of gene expression in clinically stratified tumor specimens and relate expression of each particular protein with clinical outcome. Public domain software allows researchers to examine digital images of individual histological specimens from TMAs, evaluate and score them and store the quantitative data in a relational database. TMA technology may be specifically applied to the profiling of proteins of interest in other pathophysiological conditions such as congestive heart failure, renal disease, hypertension, diabetes, cystic fibrosis and neurodegenerative disorders. This review is intended to summarize the strengths and weaknesses of TMA technology which will have an increasingly important role in the laboratories of the post-genomic era.

Offprint requests to: Dr. A. Mobasheri, Molecular Pathogenesis and Connective Tissue Research Groups, Department of Veterinary Preclinical Sciences, Faculty of Veterinary Science, University of Liverpool, Liverpool L69 7ZJ, United Kingdom. Fax: +44 151 794 4243. e-mail: a.mobasheri@liverpool.ac.uk

Key words: Tissue microarray, Gene expression, Immunohistochemistry, *In situ* hybridization, Prognostic oncology, Cancer

Introduction

The human genome project, which is nearing completion, has led to the identification of over 50,000 human genes. What remains is largely a "fill in the blanks" exercise. The challenge now is to determine the function of these genes and study their regulation in the context of the phenotype of highly specialized cell types that make up the tissues and organs of the living human organism. Biomedical scientists from different disciplines will be using the data from the human genome project to answer different types of questions. Developmental biologists and embryologists will want to know which genes are involved in embryonic development and study their spatio-temporal patterns of expression. Physiologists will be keen to exploit the genomic information to learn more about the physiology of the cells, tissues or organs of their choice. Pathologists and oncologists in the cancer research community will be involved in identification and functional analysis of the human genes involved in oncogenesis and metastasis and using the knowledge gained from the human genome project for the benefit of cancer patients. Microarray technology is useful for characterizing the expression profile of the "transcriptome" under physiological and pathological conditions particularly to measure differential gene expression, variations in the gene sequence (e.g. of mutant phenotypes) or more recently to localize entire binding sites for transcription factors (Napoli et al., 2003). The human genome project is essentially the tip of the iceberg and only the beginning of a larger quest

aimed at understanding human physiology.

Genes merely provide the biological "script" for life; it is the protein products of genes that are the real "actors", performing specific roles in cells and accomplishing the functional objectives of the genome. Fifty thousand human genes are likely to give rise to hundreds of thousands of different proteins; further levels of complexity are provided by the proteome (the protein complement of a cell) (Heal et al., 2002). Numerous types of post-translational modification (i.e. glycosylation, acylation, limited proteolysis, phosphorylation, isoprenylation) that individual proteins may undergo to modulate their function will add further levels of sophistication. The recognition that higher levels of complexity exist over and above the genome has resulted in new terminologies such as "physiomics" and "metabolomics" both of which lead to "pathomics" and new multidisciplinary projects like the "physiome project" (Table 1; McCulloch et al., 1998; Bassingthwaighte, 2000).

Proliferation of microarray technologies

High throughput screening technologies such as cDNA and oligonucleotide microarrays have proliferated in recent years making it possible to analyze the expression of thousands of genes at once from small quantities of tissue or cell samples (Schena et al., 1995). Microarray experiments have also been used to obtain expression profiles of genes of an organism growing in the mammalian tissue or in response to growth parameters (Conway and Schoolnik, 2003). This technology allows the investigator to determine phenotypic differences between two cell populations exposed to hormones, cytokines, pharmaceuticals or even a spectrum of different physiological and environmental conditions ranging from anoxia to hypoxia to normoxia. The up- or down-regulation of genes in the stimulated cell population is then compared with an unstimulated control population. Microarray technology can also be used to help understand cancer development, to improve patient treatment and management, and to identify those predisposed to developing cancer (Bubendorf et al., 1999; Cooper, 2001; Kallioniemi et al., 2001). Microarray expression patterns are currently being integrated with clinical data to identify new markers to predict biological behavior of tumors. Protein arrays are also becoming increasingly popular as the equivalent tool in the hands of the proteomics expert. However, these approaches have received little interest from histopathologists and pathologists who are primarily interested in equivalent technologies that will allow them to screen large numbers of tissue specimens for gene expression information and for discovering diagnostic and prognostic correlations. Laboratory investigators have used multiple tissue Northern and Western blots for many years for studies of gene and protein expression but these methods can be expensive, laborious and time consuming to establish. Ultimately such approaches do not allow high throughput analysis. Tissue microarrays (TMAs) are receiving considerable interest from histopathologists and pathologists and other researchers who have quickly embraced the new technology. The aim of this review is to bring readers of Histology and Histopathology and other scientists interested in post-genomic technologies up-to-date with the capabilities of this novel innovation.

Tissue MicroArrays (TMAs)

In 1997, Kononen and Kallioniemi developed the tissue microarray (TMA), an ordered array of tissue on a single glass slide (Fig. 1) that allows researchers to

Table 1. The genome and physiome projects, their associated websites and related primary publications.

PROJECT	WEBSITE	DESCRIPTION AND PRIMARY REFERENCES
The Human Genome Organisation (HUGO) and The Human Genome Project	http://www.gene.ucl.ac.uk/hugo/ http://www.sanger.ac.uk/HGP/ The human genome research programme at The Sanger Institute and other laboratories around the globe encompasses mapping, sequencing, and structural & functional interpretation.	The Human Genome Organisation aims to promote international discussion and collaboration on scientific issues and topics crucial to the progress of the world-wide human genome initiative in order that the analysis of the human genome can be achieved as rapidly and effectively as possible. It also aims to promote the scientific study of the human genome, including the identification and functional analysis of the human genes, and encourage the free flow of information unconstrained by individual, industrial or national interest. HUGO also aims to provide a global forum for addressing the scientific, medical, ethical, legal, social and commercial issues raised by the handling and use of genome knowledge. McKusick, 1989
The Physiome Project	http://www.physiome.org/	The major long-term goal of the Physiome Project is to understand and describe the human organism, its physiology and pathophysiology quantitatively, and to use this understanding to improve human health. Insight will be gained also from the physiomes of other organisms, from bacteria to mammals. McCulloch et al., 1998; Bassingthwaighte, 2000

analyze multiple tissue sections and makes direct high throughput analysis possible in highly characterized tissue samples (Kononen et al., 1998). Each TMA may contain between 50 and 600 tissue specimens. Each TMA is normally supplied with complete pathology information for every specimen on the array. This information includes subject demographics and histological diagnosis that have been confirmed by a pathologist. Data on molecular marker expression, medication and treatment outcomes may also be included when available. The expression profile of a specific target can be analyzed across various normal and diseased tissues in a single experiment, providing considerable savings in time, materials and effort. These arrays use less tissue per individual analysis and offer greater internal consistency for immunohistochemical studies. TMA may also be used for *in situ* hybridization to analyze gene expression or protein expression by immunohistochemistry directly in well characterized tissue samples. Tissue microarrays permit comparison of different techniques or multiple immunohistochemical staining of a series of slides that contain the same tissues. Since replicate samples are provided, statistically significant results can be easily obtained. TMAs will be also useful in various areas of antibody evaluation (Simon and Sauter, 2002).

Microarray applications

TMAs make it possible to analyse one gene at the DNA, RNA or protein level in hundreds of individual tissue samples (Bubendorf, 2001). Many researchers have rapidly embraced the new technology and the number of citations in Medline/PubMed using TMA technology is steadily rising. The Tissue Array Research Program (TARP) at the National Cancer Institute (http://www.nci.nih.gov/tarp), has been a front-runner in developing this technology and distributing slides to scientists at the National Institutes of Health and the international research community. The tissue microarrays have the capacity to represent every organ in the human body or in the body of any model organism. In addition, tissue microarrays may contain a wide variety of common cancers alongside normal

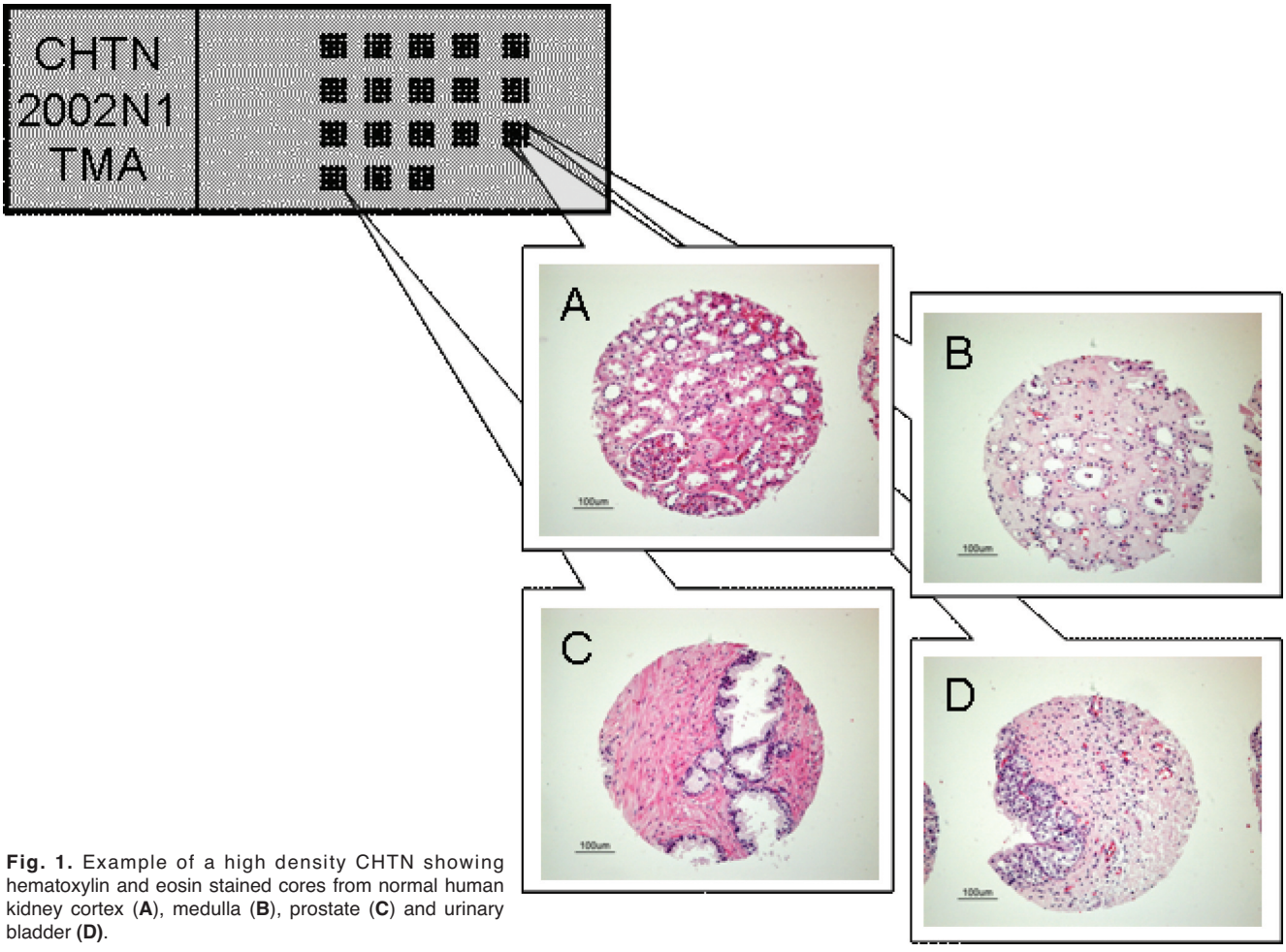


Fig. 1. Example of a high density CHTN showing hematoxylin and eosin stained cores from normal human kidney cortex (**A**), medulla (**B**), prostate (**C**) and urinary bladder (**D**).

controls (Bubendorf, 2001; Simon and Sauter, 2002). They can contain rare or obscure clinical cases, or increasingly common ones, such as breast and prostate carcinomas (Camp et al., 2000; Dhanasekaran et al., 2001; Rubin et al., 2002a,b; Varambally et al., 2003). It is even possible to apply this technology to tissues from knockout mice, veterinary species or even cultured human or animal cell lines. This technology makes it possible to obtain a complete tissue archive of a particular animal species on a single slide. From a practical perspective, these slides may be treated like any other histological section. The investigator may use them for *in situ* hybridization to detect gene expression or identify chromosomal abnormalities, or employ immunohistochemistry or immunofluorescence to detect and localize protein expression (Bubendorf, 2001). With serial sections of the master block, numerous biomarkers may be analysed (Fig. 2). TMAs may be used for basic or clinical research, prognostic histopathology or to validate potential drug targets previously identified with DNA microarrays and proteomics technology.

Tissue arrayers

Unlike DNA microarrays, which are usually made by spotting microlitre-scale sample volumes directly on

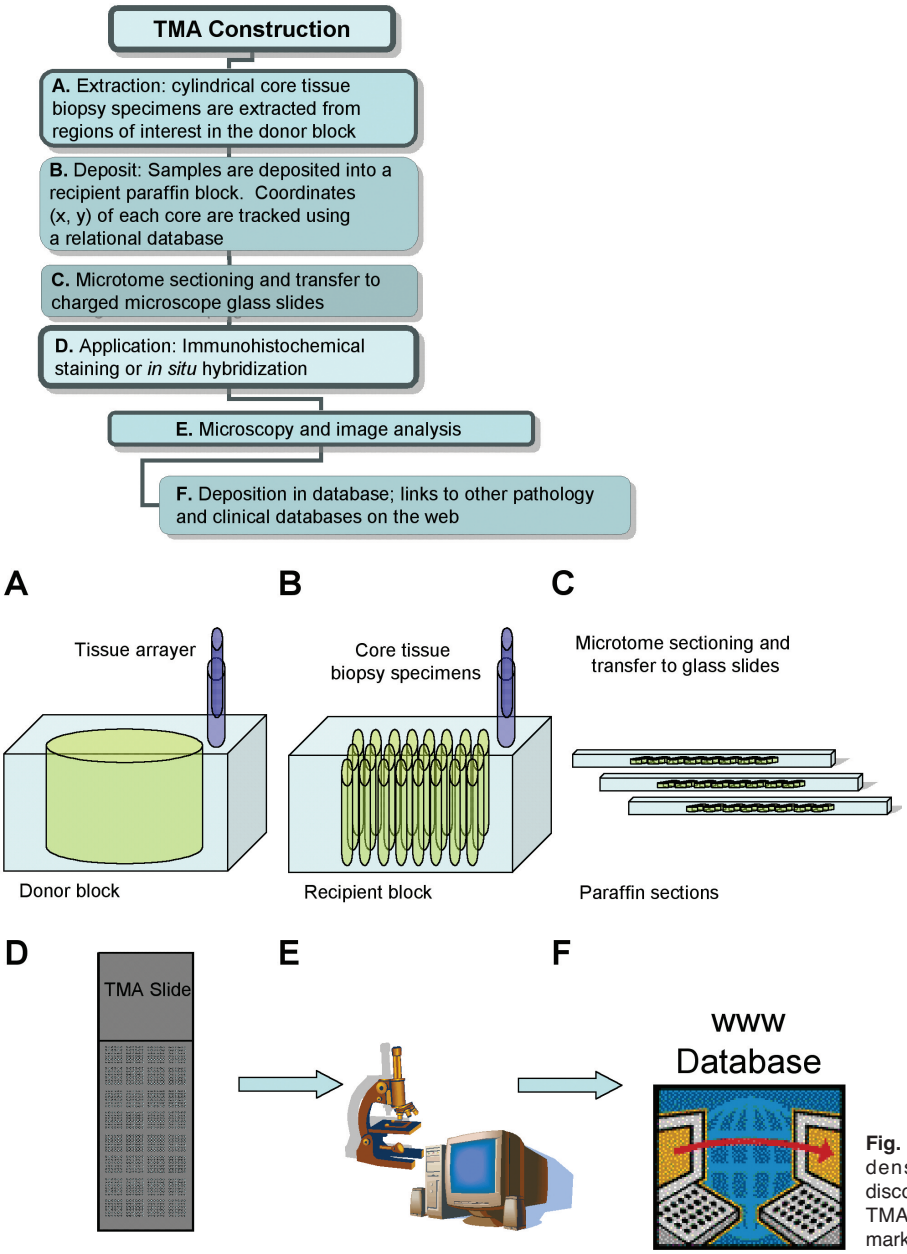


Fig. 2. TMA design and construction. The use of high density TMAs allows validation of novel gene discoveries across all human tissues by associating TMA data with clinical and pathological data novel markers of disease pathogenesis may be validated.

a charged slide, tissue microarrays are constructed in paraffin blocks commonly seen in the histopathology laboratory. Each tissue core in the array is collected as a "punch" (i.e. 0.6 to 2.0 mm in diameter) from a donor block of formalin-fixed paraffin-embedded tissue, using a precision needle (Fig. 2). A second, slightly smaller needle is used to create a hole in the recipient block. The tissue cores are then arrayed in the recipient block to produce a master block, from which hundreds of individual 4-5 μm sections may be obtained (Kallioniemi at al., 2001). Due to the difficulties associated with formalin fixation and antigen retrieval some antibodies may not work with the conventional wax embedded tissue arrays. Also, *in situ* hybridization experiments often fail with paraffin-embedded tissue samples because the nucleic acids are destroyed or fixed in such a way that they are no longer accessible to molecular probes. Therefore, some commercial companies are developing arrayers capable of handling frozen tissue samples instead of paraffin-embedded ones. Fejzo and Slamon recently described an approach to manufacture frozen TMAs for analysis of tumor RNA, DNA and proteins (Fejzo and Slamon, 2001).

Obtaining TMAs

Several commercial companies supply off-the-shelf TMAs; many of these companies offer custom services as well (Table 2). Researchers supply the tissues to be arrayed, or sometimes even the animals from which the specimens should be harvested, and the company produces a master block to customer specifications. Specimens for TMAs may also be derived from tissue archives of histopathology departments. Many medical schools maintain sample repositories, which can grow to considerable size, housing millions of archival blocks. However, for university resources there are important ethical issues to consider such as local medical ethics committee approval and patient consent. In some institutions the bureaucracy necessitates special committees to approve the use of archival material dating back several decades. If these issues are likely to be an impeding or inhibiting factor, academics may wish to consider purchasing commercial TMAs (Table 2) or approaching the National Institute of Health's Cooperative Human Tissue Network or the National Cancer Institute's TARP programme for limited supplies of TMA slides (Table 3). Some commercial companies are already collecting tissue repositories of their own through the National Clinical Genomics Initiative, which caters to both academic and private entities.

Cooperative human tissue network (CHTN)

The purpose of the CHTN at the National Institutes of Health in Bethesda is to provide researchers with a comprehensive tissue microarray of formalin fixed paraffin embedded samples that include most of the cell

Table 2. Commercial suppliers of tissue microarrays and arraying equipment.

COMPANY	WEBSITE	DESCRIPTION
Ardais	http://www.ardais.com	Clinical genomics company dedicated to enhancing and accelerating biomedical research by introducing actual human disease into the pharmaceutical discovery research process.
Ambion	http://www.ambion.com	Manufacturers of LandMark™ high and low density tissue microarrays.
BD Pharmingen	http://www.bdbiosciences.com	Suppliers of mouse normal multiple tissue arrays.
Beecher Instruments	http://www.beecherinstruments.com	Beecher Instruments develops microarray technologies for high-throughput genomic and proteomic research. Beecher also offers automated and manual tissue microarray instruments and accessory tissue arrayer products.
BioCat	http://www.biocat.de	Antibody arrays for cytokine expression profiling, multiple tissue protein Western blots, multiple tissue protein arrays and multiple tissue arrays for immunohistochemistry and *in situ* hybridization. Biocat also offers whole body tissue arrays made from human normal tissues and system and organ specific arrays (i.e. CNS, cartilage and synovitis tissue arrays, fetal and tumor tissue arrays).
Chemicon International	http://www.chemicon.com	Manufacturers of advanced tissue arrayers, normal mouse and rabbit tissue arrays.
Clinomics Biosciences	http://www.clinomicsbio.com	Suppliers of Clinomics™ tissue microarray systems.
Imgenex	http://www.imgenex.com	Manufacturers of Tissue Array/ Histo-Array™ containing 60 different organs of the human body, or 60 different cases of specific human cancers.
InnoGenex	http://www.innogenex.com	Suppliers of mouse, rat, and human tissue microarrays for *in situ* localization of proteins and for gene expression.
Invitrogen	http://www.invitrogen.com	Suppliers of VastArray™ tissue arrays on standard glass microscope slides containing 600μm core tissue samples taken from normal human and mouse organs.
Zymed Laboratories	http://www.zymed.com	Manufacturer's of MaxArray multi-species tissue arrays containing tissue samples arrayed on Superfrost Plus microscope slides.

Table 3. Non-commercial suppliers of tissue microarrays.

INSTITUTION	CONTACT DETAILS	DESCRIPTION
Tissue Array Research Program (TARP) at the National Cancer Institute, Charlottesville, VA, USA	TARP, The National Cancer Institute, 566 Dulles Bldg., 3400 Spruce Street, Philadelphia, PA 19104, USA, FAX: 215-614-0251 Website: http://www.nci.nih.gov/tarp	The TARP program is a collaborative effort between the National Cancer Institute and The National Human Genome Research Institute. The multi-tumor tissue microarrays are provided only for research and not for diagnostic purposes.
Cooperative Human Tissue Network of The National Cancer Institute, The National Institutes of Health, Bethesda, MD, USA	CHTN, Mid-Atlantic Division, The National Cancer Institute, University of Virginia Health System, P.O. Box 800423, Charlottesville, VA 22908, USA, FAX: (434) 924-9438. Website: http://faculty.virginia.edu/chtn-tma/home.html	To provide researchers with a tissue microarray of formalin fixed paraffin embedded samples that includes most of the cell types present in the human body. Microarrays are intended for research and not for diagnostic purposes.

types present in the human body. The CHTN also provides researchers with inexpensive "test" tissue microarrays of a limited number of formalin fixed paraffin embedded samples that can be used to titrate assay parameters prior to use of more comprehensive tissue microarrays. These test arrays are selected to contain lymphoid, epithelial and stromal tissues (i.e. spleen, colonic mucosa, endometrium, liver and uterine smooth muscle (Fig. 3). The tissue samples provided on the more comprehensive arrays (i.e. 66 tissue types) are non-neoplastic adult tissue obtained from surgical resection specimens (Fig. 4). All tissues selected are fixed within one hour of removal from the donors. The fixative used is buffered zinc formaldehyde (3.7% formaldehyde), although there may be some exceptions (i.e. some tissues may be derived from benign tumors) due to the limiting size and limited availability of normal tissue or because of the difficulty in sampling normal tissue. The tissue samples from the central nervous system are not always fresh since they may be obtained from autopsy specimens up to 36 hours after death. The CHTN tissue microarrays are only provided to the requesting investigator for research purposes and are not intended for diagnostic purposes. All the samples are anonymized and no clinical information is available about the tissue donors other than the age and sex data that accompany the arrays, usually in the form of excel spreadsheets (Fig. 5). Guide sheets that include representative histologic figures can also be downloaded in Microsoft Excel format from the CHTN TMA website: http://faculty.virginia.edu/chtn-tma.

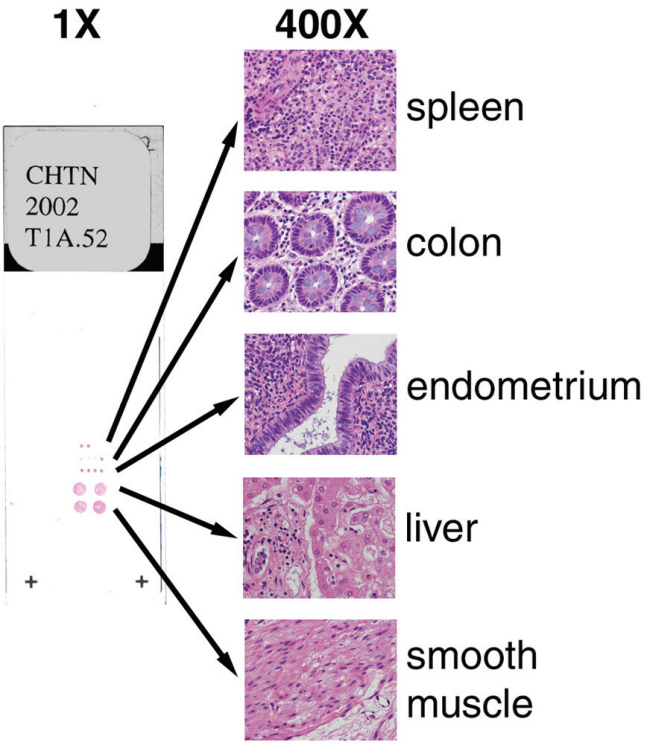


Fig. 3. Test microarrays of normal human tissues provide researchers with an inexpensive TMA of a limited number of formalin fixed paraffin embedded samples that can be used for optimization of immunohistochemical and *in situ* hybridisation parameters prior to use of more comprehensive tissue microarrays.

Normal TMA Design

Each tissue type is sampled several times with 0.6 mm needle cores in the original array design and each array block is serially sectioned at 4 micron thickness (Simon and Sauter, 2002). The histologic sections are placed on charged glass slides and at regular intervals, sections are stained and examined by a pathologist for quality assurance (QA) purposes. Each specimen in the TMA has an exact X-Y position and the precise arrangement of samples facilitates interpretation of staining and serves as an ideal basis for automated analysis (Bubendorf et al., 2001). The number of tissue spots in which the desired tissue types resides may vary from section to section. In cases where QA results indicate that a particular target tissue is not represented in the histologic sections of the TMA, an auxiliary TMA is constructed (using larger tissue cores) to supplement the tissue present in any given main TMA. TMAs can also be made from experimental tissues such as cell lines and xenografts (Simon and Sauter, 2002). Cell-line arrays of formalin fixed and paraffin embedded cell

Table 4. List of normal tissues available on the CHTN TMA codenamed CHTN2002N1.

CODE	TISSUE	CODE	TISSUE	CODE	TISSUE
BE	breast, epithelium	GIE	esophagus, squamous mucosa	NCPG	cerebellar cortex, purkinje/granular cells
CASM	aorta, smooth muscle	GIGA	gastric mucosa, antral	NCWM	white matter (subcortical)
CHM	heart, myocardium	GIGO	gastric mucosa, oxyntic	NPAG	autonomic ganglia and nerves, intestinal
CLE	lymphatic endothelium	GISI	small intestine, mucosa	NPPN	peripehral nerve
CSMA	small muscular artery	GME	epididymis	OSSG	salivary gland (parotid)
CSV	small vein (intestine)	GMP	prostate	OSTSE	tonsil, squamous epithelium
EAGC	adrenal gland, cortex	GMST	seminifeours tubules	PAM	amniotic membrane
EAGM	adrenal gland, medulla	GMSV	seminal vesicle	PV	placenta, villi
EPA	pituitary, anterior	HPG	gallbaldder	RA	alveoli
EPAD	parathyroid adenoma	HPL	liver	RBE	bronchus, epithelium
EPP	pituitary, posterior	HPP	pancreas	SSE	skin, squamous epithelium
ET	thyroid	LLN	lymph node	SST	subepidermal tissue
GFEC	ectocervix	LMALT	mucosa assoc. lymphoid tissue, appendix	STAB	adipose tissue, breast
GFEN	endocervix	LS	spleen	STCA	cartilage, articular
GFES	endometrium, secretory	LT	thymus	STCB	cartilage, bronchial
GFFT	fallopian tube	LTL	tonsil	STS	synovium
GFOCL	ovary, corpus luteum	NCCC	cerebral cortex	STSKM	skeletal muscle
GFOE	ovary, epithelium	NCCP	choroid plexus	STSMI	smooth muscle, intestine
GFOO	ovary, 1° oocyte	NCE	ependymal cells	STSMU	smooth muscle, uterus
GFOS	ovary, stroma	NCH	hippocampus	UBTE	bladder, transitional epithelium
GIA	anus, mucosa	NCM	meninges	UKC	kidney, cortex
GIC	colon, mucosa	NCMN	motor neurons (spinal cord)	UKM	kidney, medulla

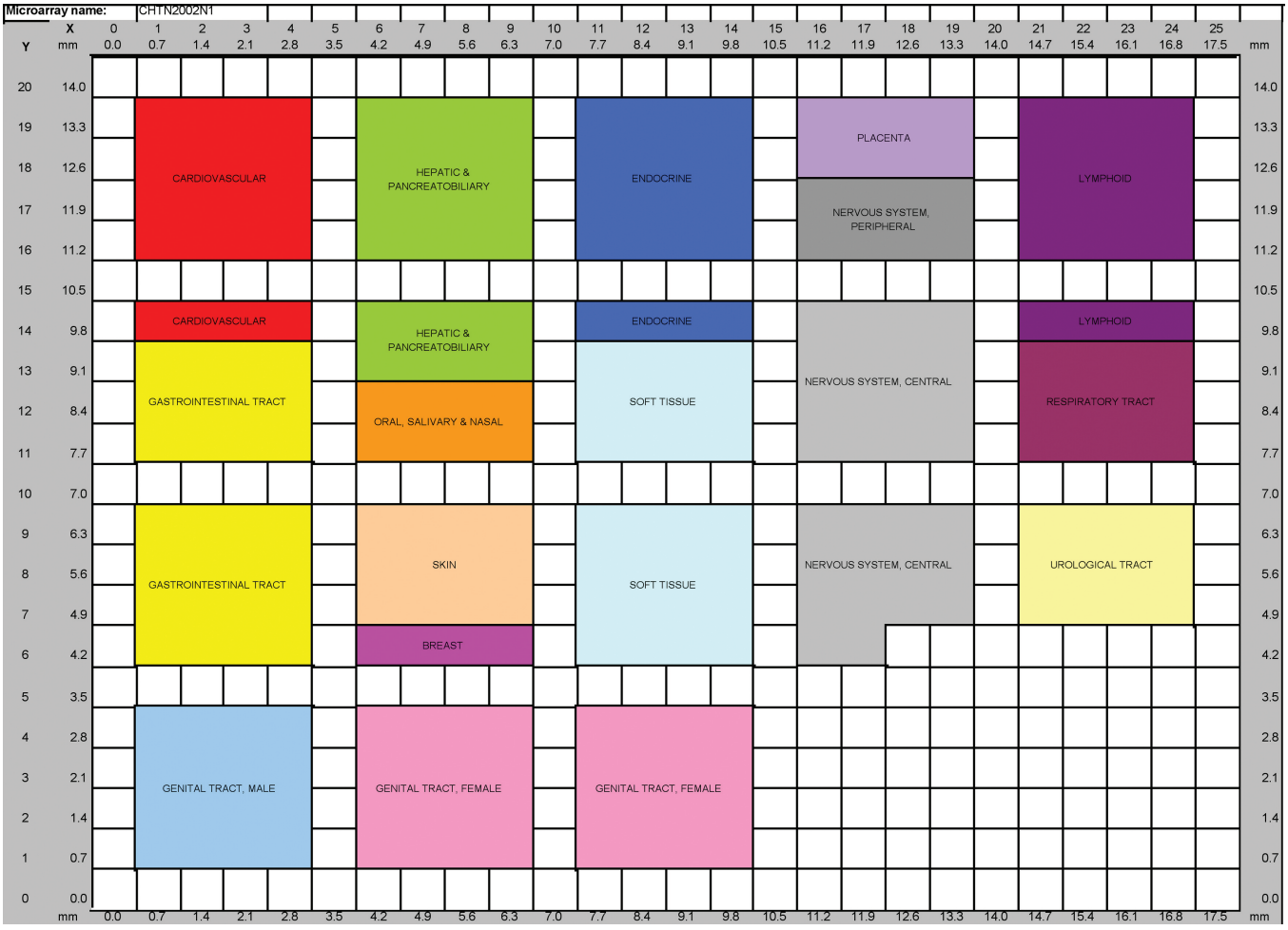


Fig. 4. Tissue microarray of formalin fixed paraffin embedded samples that includes 66 normal tissue types present in the human body. This microarray which is available from the Cooperative Human Tissue Network (CHTN) is codenamed CHTN2002N1. For a list of tissues corresponding to the codes see Table 4.

pellets have also been prepared (Simon and Sauter, 2002).

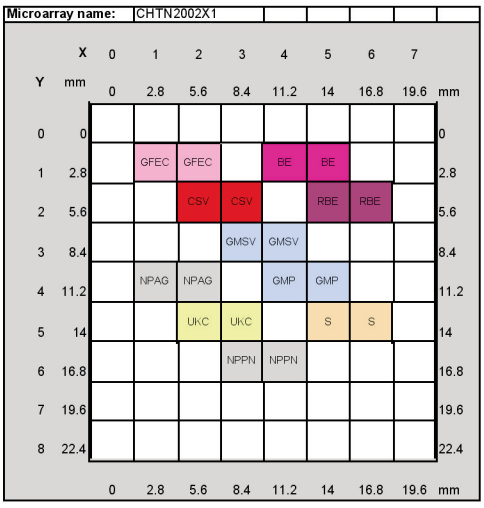
Common problems with normal TMAs

Tissue cores used for the normal TMA design are of various lengths. Thus at deeper sections, some cores will be exhausted while others will remain intact. In addition, some tissue spots may be lost during the process of transferring the TMA section to the charged glass slide. Another important variation that may be encountered is lack of uniformity; although TMA manufacture is guided by a histologic section that represents the surface of the donor tissue, this target tissue may not be uniformly represented in the deeper sections of the tissue. This is due to the variability inherent in tissue architecture and is a common problem with small and delicate anatomical structures (e.g. breast ducts and lobules). Particularly small core samples of a heterogeneous tissues e.g. a large tumor may not be representative. Therefore it should be kept in mind that TMA is more suitable as a population level research tool than intended for clinical diagnoses of individual cases. Punching multiple small cores from different regions of tissue captures the heterogeneity of such tumors. The representative microscopic image of the target tissue may not precisely match the tissue spot on the TMA because representative images may have been taken from a single spot from a single QA section from a single array. Four different replicate TMA blocks are usually made for this series, each of which has different tissue cores. Even the same tissue core at a deeper section may not exactly match a more superficial section due to the variability in the 3-dimensional tissue architecture. Although antigen retrieval methods (boiling, microwave heating and pressure cooking) may be used on these sections, some tissue spots may easily become detached during antigen retrieval.

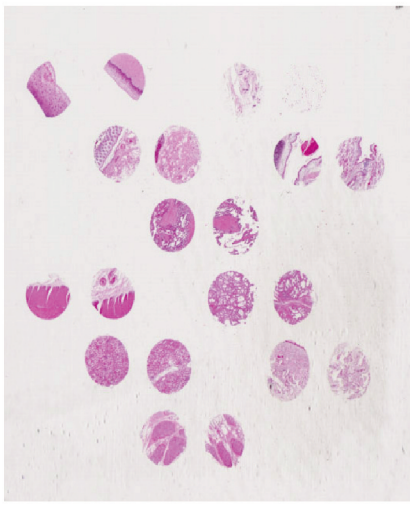
Multi-tumor tissue microarrays

The National Cancer Institute's TARP program provides limited supplies of TMA slides to investigators for a nominal fee per slide plus shipping charges. These

A



B



C

Tissue	Code	Age	Sex
BREAST			
breast, epithelium	BE	60	F
CARDIOVASCULAR			
small vein (intestine)	CSV	85	M
GENITAL TRACT, FEMALE			
ectocervix	GFEC	52	F
GENITAL TRACT, MALE			
prostate	GMP	59	M
seminal vesicle	GMSV	65	M
NERVOUS SYSTEM, PERIPHERAL			
autonomic ganglia & nerves, intestinal	NPAG	52	M
peripheral nerve	NPPN	62	M
RESPIRATORY TRACT			
bronchus, epithelium	RBE	73	M
SKIN			
skin	S	62	M
UROLOGICAL TRACT			
kidney, cortex	UKC	47	M

Fig. 5. Auxiliary normal tissue microarray available from the Cooperative Human Tissue Network (CHTN) codenamed CHTN2002X1. **Panel A** shows the TMA slide layout and the tissue codes. A representative hematoxylin and eosin stained section is shown in **panel B** and **panel C** represents the tissue list donor age and sex data that usually accompany the arrays in a Microsoft Excel spreadsheet file.

TMAs consist of two rows of normal tissues and up to 18 panels of various types of tumors (Fig. 6). These arrays will allow investigators to examine the expression of proteins of interest in up to 500 different normal and tumor specimens by immunohistochemistry. In combination with DNA microarrays where one tumor is analysed for the expression of thousands of genes at a time, tissue microarrays can be used for the simultaneous analysis of one protein in hundreds of different tumors ("multi-tumor TMA"). This technology enables investigators to find novel prognostic markers of specific tumors by investigation of expression of genes in many tumor samples under the same conditions (Moch et al., 2001). TMA technology is suitable for high-throughput molecular epidemiologic studies of tumors. In comparison with conventional tissue sampling, TMA technology supports more representative scoring of molecular markers expressed by tumors (Camp et al., 2000). TMAs have also been used as "progression TMAs" containing progression stages of various tumors to study cancer development and progression (Bowen et al., 2000; Simon and Sauter, 2002). "Prognosis TMAs" contain samples from tumors with available clinical follow-up data and endpoints (Moch et al., 1999; Simon and Sauter, 2002).

Data storage and analysis

TMAs present histopathologists and cell biologists with significant challenges in data management and bioinformatics. TMA users need to keep track of detailed clinical and experimental data, and they require an easy way to access the visual data associated with the experiments. Each new antibody that researchers use to probe a given array not only adds to that array's value, but also increases the data's complexity. Software systems have been developed to deal with the image archiving problem. Software packages in the public domain allow researchers to examine digital images of individual histological specimens, such as tissue cores

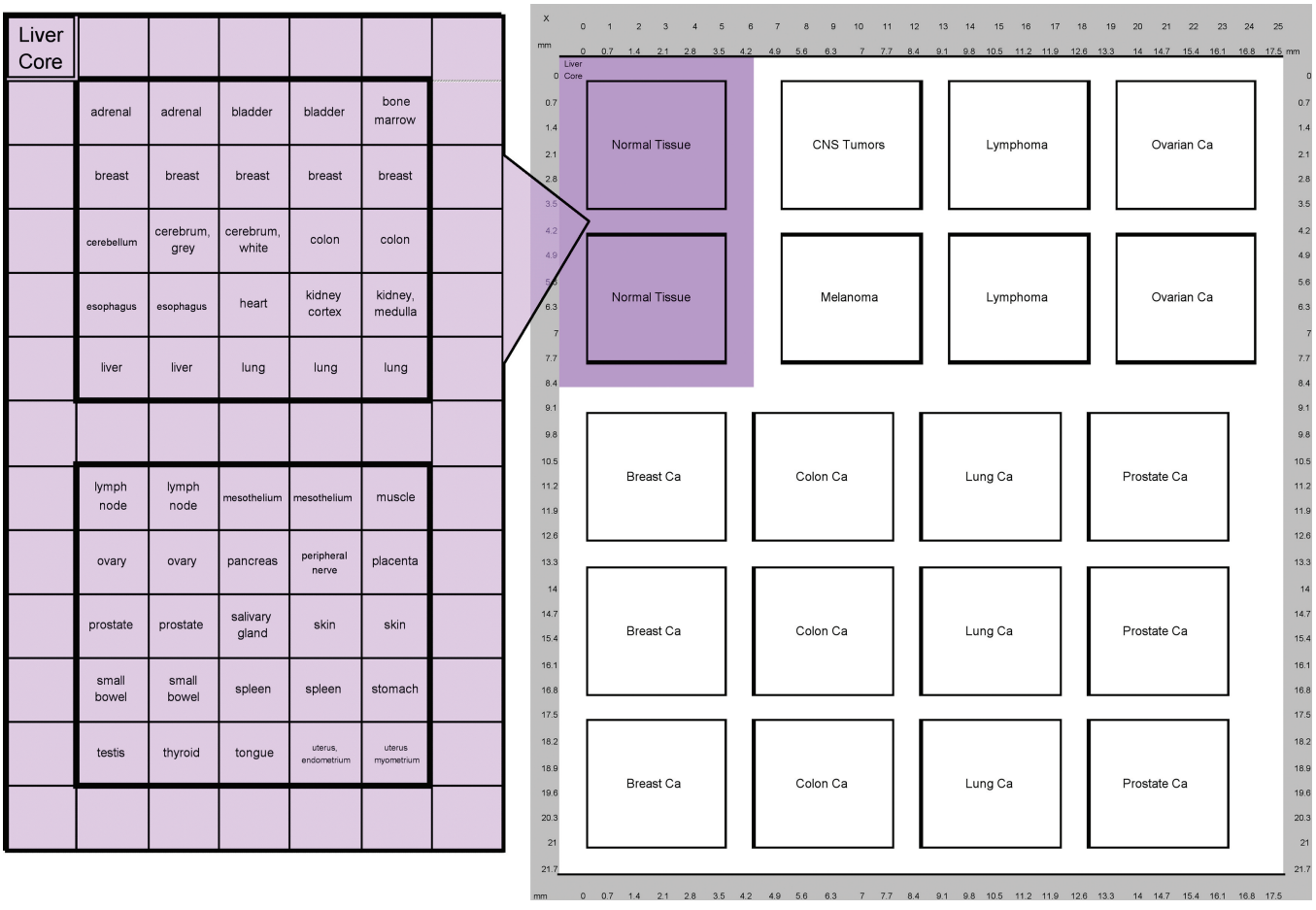


Fig. 6. Illustration of the TARP 4 multi-tumor tissue microarray originally available from the National Cancer Institute which contains normal and tumor specimens on a single slide. Details and orientation of normal tissues are also represented on the enlarged panel on the left of the main TARP 4 microarray slide. (see http://resresources.nci.nih.gov/tarp/about.cfm).

from a TMA, evaluate and score them, and store all the data in a relational database. Liu and co-workers (2002) have developed a comprehensive system for high-throughput analysis and storage of TMA immunostaining data, using a combination of commercially available systems and newly developed software applications such as: TMA-Deconvoluter (and Stainfinder, a novel web-based program both available at http://genome-www.stanford.edu/TMA). Staining results are recorded directly into a Microsoft Excel worksheet and are reformatted by a program called TMA-Deconvoluter (a series of Excel macros) into a format suitable for hierarchical clustering analysis or other statistical analysis. These can then be read by conventional data analysis tools like Cluster and TreeView, both of which are freely available public domain programs that can be obtained at http://rana.lbl.gov/EisenSoftware.htm. Cluster runs a hierarchical cluster analysis on the TMA data, helping users to interpret the highly complex datasets obtained from TMAs stained with large numbers of antibodies, and TreeView allows researchers to browse the clustered data. Hierarchical clustering analysis is a powerful means of assessing relatedness within groups of tumors (based on their immunostaining with a panel of antibodies). Other analyses, such as generation of survival curves, construction of Cox regression models, or assessment of intra- or interobserver variation, can also be done readily on the reformatted data. Finally, Stainfinder is a Web interface that links the clustered TMA data to an online image database, allowing rapidly re-evaluation of the data and comparison of different stains on the same core.

Data interpretation

To produce such an image database, all images of the tissue cores must be captured in digital format. Sophisticated automated slide scanners are available but the scoring process may be performed by any computer (by scanning the slides) or by eye (as performed by a pathologist). However, both scoring processes, particularly the latter are inherently subjective and imprecise. Whereas DNA biochip information is analyzed by a computer, which provides a value over a large dynamic range, many pathologists score manually using a four-point scale: negative, weak positive, strong positive, or no data (i.e. 0 to 3+). An automated microscopy device and software package has been developed called Aqua (Camp et al., 2002). This software can analyze a slide and output a value that indicates the level of expression of a particular protein within a user-defined subcellular compartment.

Concluding remarks

The driving force behind the development of TMAs was to take the genomics approach already established for DNA and protein microarrays and apply it to the study of clinical tissue samples. TMAs are a significant advance over previous attempts to put multiple samples in a single paraffin block. There are many benefits to using TMAs; one is the ability to screen large numbers of cases in a single immunohistochemical staining run, thereby minimizing day-to-day experimental variability in immunohistochemical staining. TMAs also decrease the cost of conducting immunohistochemical studies, and increase the numbers of studies that can be analyzed on small pieces of tissue, by using small cores of tissue rather than cutting sections of every block for each study. DNA microarray technology can be combined with TMA technology to help understand fundamental biological phenomena such as tissue specific gene expression. Thus far, we have successfully used TMA technology to study the expression of the aquaporin 1 (AQP1) water channel in normal human tissues (Mobasheri and Marples, 2003), and in pathologies of human articular cartilage (Trujillo et al., 2004). A marriage of these two techniques has been applied in the field of oncology to study cancer development and metastasis and identify novel markers that may predict the aggressive behaviour of tumors. If properly used, these technologies have the potential for applying clinical genomics and "pathomics" in practice. There is still a vast amount of development work that still needs to be done and TMA technology will have an increasingly important role in the laboratories of the post-genomic era.

Acknowledgements. The authors thank Miss Rachel Floyd (Department of Physiology, University of Liverpool) for helpful comments on the manuscript. We would also like to acknowledge Dr. Stephen M. Hewitt (TARP Laboratory, Molecular Profiling Initiative, Advanced Technology Center, Nationqal Cancer Institute, National Institute of Health, Bethesda, MD USA) for his support and assistance.

References

Bassingthwaighte J.B. (2000). Strategies for the physiome project. Ann. Biomed. Eng. 28, 1043-1058.

Bowen C., Bubendorf L., Voeller H.J., Slack R., Willi N., Sauter G., Gasser T.C., Koivisto P., Lack E.E., Kononen J., Kallioniemi O.P. and Gelmann E.P. (2000). Loss of NKX3.1 expression in human prostate cancers correlates with tumor progression. Cancer Res. 60, 6111-6115.

Bubendorf L. (2001). High-throughput microarray technologies: from genomics to clinics. Eur. Urol. 40, 231-238.

Bubendorf L., Kononen J., Koivisto P., Schraml P., Moch H., Gasser T.C., Willi N., Mihatsch M.J., Sauter G. and Kallioniemi O.P. (1999). Survey of gene amplifications during prostate cancer progression by high-throughout fluorescence *in situ* hybridization on tissue microarrays. Cancer Res. 59, 803-806.

Bubendorf L., Nocito A., Moch H. and Sauter G. (2001). Tissue microarray (TMA) technology: miniaturized pathology archives for high-throughput *in situ* studies. J. Pathol. 195, 72-79.

Camp R.L., Charette L.A. and Rimm D.L. (2000). Validation of tissue microarray technology in breast carcinoma. Lab. Invest. 80, 1943-

1949.

Camp R.L., Chung G.G. and Rimm D.L. (2002). Automated subcellular localization and quantification of protein expression in tissue microarrays. Nat. Med. 8, 1323-1328.

Conway T. and Schoolnik G.K. (2003). Microarray expression profiling: capturing a genome-wide portrait of the transcriptome. Mol. Microbiol. 47, 879-889.

Cooper C.S. (2001). Applications of microarray technology in breast cancer research. Breast Cancer Res. 3, 158-175.

Dhanasekaran S.M., Barrette T.R., Ghosh D., Shah R., Varambally S., Kurachi K., Pienta K.J., Rubin M.A. and Chinnaiyan A.M. (2001). Delineation of prognostic biomarkers in prostate cancer. Nature 412, 822-826.

Fejzo M.S. and Slamon D.J. (2001). Frozen tumor tissue microarray technology for analysis of tumor RNA, DNA, and proteins. Am. J. Pathol. 159, 1645-1650.

Heal J.R., Roberts G.W., Raynes J.G., Bhakoo A. and Miller A.D. (2002). Specific interactions between sense and complementary peptides: the basis for the proteomic code. Chembiochem. 3, 136-151.

Kallioniemi O.-P., Wagner U., Kononen J. and Sauter G. (2001). Tissue microarray technology for high-throughput molecular profiling of cancer. Hum. Mol. Genet. 10, 657-662.

Kononen J., Bubendorf L., Kallioniemi A., Barlund M., Schraml P., Leighton S., Torhorst J., Mihatsch M.J., Sauter G. and Kallioniemi O.P. (1998). Tissue microarray for high-throughput molecular profiling of tumor specimens. Nat. Med. 4, 844-847.

Liu C.L., Prapong W., Natkunam Y., Alizadeh A., Montgomery K., Gilks C.B. and van de Rijn M. (2002). Software tools for high-throughput analysis and archiving of immunohistochemistry staining data obtained with tissue microarrays. Am. J. Pathol. 161, 1557-1565.

Mobasheri A. and Marples D. (2003). Expression of the aquaporin 1 (AQP1) water channel in normal human tissues: a semi-quantitative study using tissue microarray technology. Am. J. Physiol. (in press). 2003: 00408.2003.

McCulloch A., Bassingthwaighte J.B., Hunter P., and Noble D. (1998). Computational biology of the heart: from structure to function. Prog. Biophys. Mol. Biol. 69, 153-155.

McKusick V.A. (1989). The human genome organisation: History, purposes, and membership. Genomics 5, 385-387.

Moch H., Kononen J., Kallioniemi O.-P. and Sauter G. (2001). Tissue microarrays: what will they bring to molecular and anatomic pathology? Adv. Anat. Pathol. 8, 14-20.

Moch H., Schraml P., Bubendorf L., Mirlacher M., Kononen J., Gasser T., Mihatsch M.J., Kallioniemi O.P. and Sauter G. (1999). High-throughput tissue microarray analysis to evaluate genes uncovered by cDNA mircoarray screening in renal cell carcinoma. Am. J. Pathol. 154, 981-986.

Napoli C., Lerman L.O., Sica V., Lerman A., Tajana G. and de Nigris F. (2003). Microarray analysis: a novel research tool for cardiovascular scientists and physicians. Heart 89, 597-604.

Rubin M.A., Zhou M., Dhanasekaran S.M., Varambally S., Barrette T.R., Sanda M.G., Pienta K.J., Ghosh D. and Chinnaiyan A.M. (2002a). Alpha-methylacyl coenzyme A racemase as a tissue biomarker for prostate cancer. JAMA 287, 1662-1670.

Rubin M.A., Dunn R., Strawderman M. and Pienta K.J. (2002b). Tissue microarray sampling strategy for prostate cancer biomarker analysis. Am. J. Surg. Pathol. 26, 312-319.

Schena M., Shalon D., Davis R.W. and Brown P.O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270, 467-470.

Simon R. and Sauter G. (2002). Tissue microarrays for miniaturized high-throughput molecular profiling of tumors. Exp. Hematol. 30, 1365-1372.

Trujillo E., Gonzalez T., Marín R., Martín-Vasallo P., Marples D. and Mobasheri A. (2004). Human articular chondrocytes, synoviocytes and synovial microvessels express aquaporin water channels; upregulation of AQP1 in rheumatoid arthritis. Histol. Histopathol. (in press).

Varambally S., Dhanasekaran S.M., Zhou M., Barrette T.R., Kumar-Sinha C., Sanda M.G., Ghosh D., Pienta K.J., Sewalt R.G., Otte A.P., Rubin M.A. and Chinnaiyan A.M. (2003). The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature 419, 624-629.

Accepted October 20, 2003