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Tissue Microarrays in Biomedical Research

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Abstract

Recent studies in molecular biology and proteomics have identified a significant number of novel diagnostic, prognostic, and therapeutic disease markers. However, validation of these markers in clinical specimens with traditional histopathological techniques involves low throughput and is time consuming and labor intensive. Tissue microarrays (TMAs) offer a means of combining tens to hundreds of specimens of tissue onto a single slide for simultaneous analysis. This capability is particularly pertinent in the field of cancer for target verification of data obtained from cDNA microarrays and protein expression profiling of tissues, as well as in epidemiology-based investigations using histochemical/immunohistochemical staining or in situ hybridization. In combination with automated image analysis, TMA technology can be used in the global cellular network analysis of tissues. In particular, this potential has generated much excitement in cardiovascular disease research. The following review discusses recent advances in the construction and application of TMAs and the opportunity for developing novel, highly sensitive diagnostic tools for the early detection of cardiovascular disease.

Keywords: Tissue microarrays, high-throughput tool, diagnostic tools, cardiovascular disease, translational medicine

Introduction

High-throughput tools used to explore the global expression of proteins within tissues have become essential for understanding the gap between genomic profiling and cellular behavior. Most pathological tissue specimens are routinely formalin fixed and paraffin-embedded before histological evaluation. Such specimens are available in nearly unlimited numbers in hospitals or research institutes, and most have associated clinical information. In order to achieve a high level of statistical significance, hundreds or thousands of specimens must be analyzed. However, large-scale tissue analyses have traditionally been inconvenient and slow because pathology is a low-throughput, time-consuming technique that relies on observation and description. This obstacle was compounded by the completion of the human genome, which introduced a new level of correlation of disease with biology (Pennisi, 2003). In order to handle the demand for large sample sets of well-characterized human tissues, researchers are turning to tissue microarrays (TMAs) (Sauter et al., 2003). The following article describes the current status of TMA research, including techniques, applications, and data analysis. In addition, we discuss the application of TMAs in cardiovascular disease research based on previously published data.

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Abbreviations: TMA, Tissue microarray; IHC, Immunohistochemistry; ISH, *in situ* hybridization; FISH, Fluorescence *in situ* hybridization; H&E, Hematoxylin and eosin

TMA Construction

A well-designed TMA can circumvent the need to perform the same experiment repeatedly, and reduces the variability between experiments performed in multi-batch mode. Notably, the TMA was not introduced *de novo*; rather, it is an improvement on previous methodologies. The best-known precursor is the "sausage" block method described in 1986 by Hector Battifora (Battifora, 1986). This platform lacked high density, and more importantly, it lacked an organized grid of tissue that enabled simple identification of cores to match the donor tissue. Kononen *et al* (1998) addressed these problems by developing a platform that used hollow needles in a stationary apparatus.

Tissue collection and processing

The stepwise construction of a TMA begins with the ethical collection of tissue. It is essential to use approved protocols for handling animal tissue. The regulations for the use of human tissue vary from country to country. In some countries, basic approval is given by an institutional review board, which provides guidelines for the use of the tissue.

Many human TMAs have been constructed using anonymous tissue, and typically lack clinical follow-up data. These do not require approval for use by the individual donor. However, TMAs with complete clinical and epidemiology annotation offer greater potential benefit.

The majority of the tissues used in human TMAs are derived from specimens obtained during surgery (*e.g.*, organ, biopsy) or autopsy. For optimal protein or nucleic acid preservation, the tissue should be frozen or fixed directly after surgery. Autopsy-derived tissue is at significant risk for autolytic defects, which can significantly affect immunohistochemistry (IHC) or *in situ* hybridization (ISH) results. As a result, the use of autopsy-derived tissue in TMAs is limited; however, for some tissues (*e.g.*, normal brain and heart), it is the only option. Human TMAs may be obtained from the Cooperative Human Tissue Network (CHTN) of the National Cancer Institute. These TMAs contain formalin-fixed paraffin-embedded samples of 66 non-neoplastic adult tissues obtained from surgical resection specimens, obtained within 1 hour of surgical removal from anonymous donors.

In general, formalin-fixed, paraffin-embedded tissue blocks are used to construct TMAs. In special applications (*e.g.*, animal, xenograft, and cell line arrays), 70% ethanol may be used as a fixative (Hakimian and Korn, 2004). Fixatives that contain acid or heavy metals (B5) should be avoided whenever possible. These may result in brittle tissues and variable damage to proteins and nucleic acids from the tissue block. Tissues should be decalcified with a fixative saturated with EDTA rather than a strong acid.

Array design

Donor tissue core needles are available in four standard sizes: 0.6, 1.0, 1.5, and 2.0 mm. The core size correlates with the number of cores that can be placed in a single block. The practical limits are 500 cores with 0.6-mm needles and 80 cores with 2.0-mm needles. Importantly, the best approach in array design is to fill the array. We recommend maximizing the number of samples, or in the absence of samples, to over-sample or use larger cores. The approach taken must be balanced by the need to construct an array that can actually be analyzed. A single slide with 1,000 cores distributed across it without orienting markers or open space is extremely difficult to analyze.

Appropriate representation of the tissue arrayed is essential. Many researchers prefer to use whole sections of tissue for study; indeed, this is generally the case in tumor sampling (Sauter et al., 2003). It is rather challenging to determine what the appropriate representation of a tumor will be to replicate the staining pattern of a particular antibody in the section, especially in the absence of extensive prior studies (Torhorst et al., 2001). In fact, it can be argued that matching the staining pattern of whole-tissue sections is not the correct end point; rather, it should be sampling that produces a more scientifically valid prognostic significance (Fergenbaum et al., 2004; Rubin et al., 2002).

A key factor in the design of an array is the homogeneity of the donor tissue. Confluent zones of tumor or less complex normal tissues such as liver can easily be represented with a small needle. However, small targets, such as ductal carcinoma *in situ* of the breast or the glomeruli of the kidney, require the use of larger diameter needles to obtain the targeted tissue. All of the tissues represented on the TMAs that are available from the CHTN have been obtained commercially and grouped into the following categories: cardiovascular, respiratory, gastrointestinal, hepatic and pancreatobiliary, oral, salivary and nasal, mammary, endocrine, genital tract, central and peripheral nervous systems, urinary tract, skin, cartilage, and synovium. In addition to collecting these normal tissue blocks, researchers may obtain abnormal cardiovascular tissue blocks from a variety of animal models.

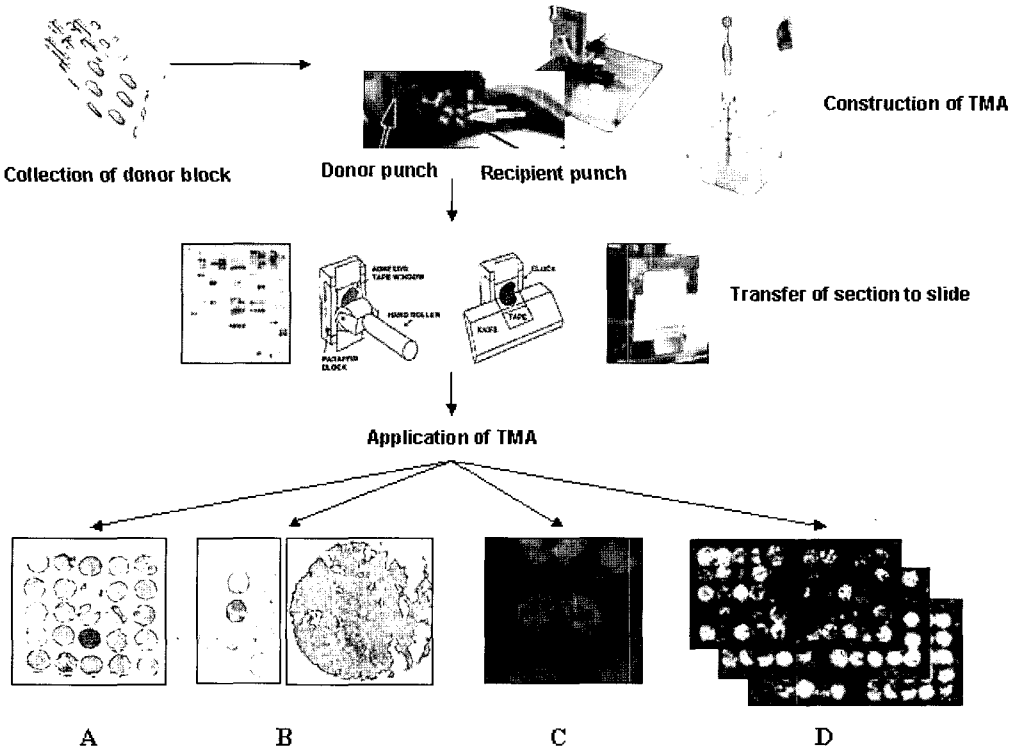

Figure 1. TMA construction and application. Useful paraffin blocks and slides of possible donor tissue are selected from the archive. A hematoxylin and eosin stained section overlaid on the surface of the donor block is used to guide sampling from representative sites in the tissue. A paraffin block core is removed from the fixed recipient block. The tissue core is transferred into a pre-made hole at defined array coordinates in the recipient block. This block can be cut in the fashion typical for any paraffin block. With the tape-transfer system, a special tape is placed directly on the block within the microtome. After cutting, the tape with the section is then positioned on a slide coated with artificial resin. Finally, the slide is treated with a special solvent for tape take-away, leaving the section alone on the slide for further processing. The sections transferred to glass slides can be applied to histochemistry (hematoxylin and eosin staining; A), IHC with various antibodies (*e.g.*, p53 monoclonal antibody; B) or ISH analysis (*HER2*; C). Another approach to detect proteins is the novel transfer method developed by our group. The tissue-section (5 μm) is first deparaffinized, followed by exposure to enzymes. A membrane stack (up to 10 membranes) is then placed over the tissue, and a transfer buffer is added. This "sandwich" is then exposed to heat to achieve protein transfer. The transferred proteins are detected on the membranes by conventional immunoblotting methodologies. This novel transfer method makes archival paraffin embedded tissue available for molecular profiling in a rapid and quantifiable manner (D).

A common failure of some TMAs is the lack of appropriate controls. Although many normal tissues are difficult to present on a TMA, there are appropriate methodological alternatives, including using whole sections or alternative tissues. Tissue should be organized in a logical fashion.

TMA construction

The first step of TMA construction is to review the hematoxylin and eosin stains of the original sections to determine regions of interest (Figure 1). After designing the array, cylinders of tissue are punched from the donor block using a hollow needle. The choice of paraffin is significant. It must be soft enough at room temperature to punch holes in without cracking the block (Dennis et al., 2003). Once arraying is complete, the surface of the recipient block should be flattened. This is best accomplished by warming the recipient block in an oven at 37°C for several hours.

One commonly used tissue-arraying instrument is manufactured by Beecher Instruments (San Prairie, WI). The instrument consists of a pendulum arm with two hollow needles of

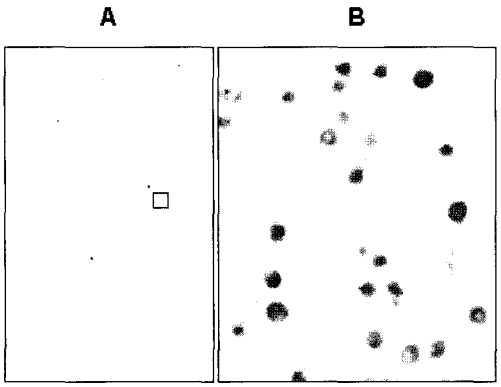

Figure 2. Tissue microarraying of cultured cells. This array consists of 60 cancer cell lines used by the NCI to screen chemotherapeutic agents. The preparation includes a fixation step, with cells then dispersed in agarose agar (matrix between the cells seen at the higher magnification). (A) After standard processing agarose pellets are placed in the paraffin block as human tissue, which then becomes "ready to punch". This array is mostly used to perform ICH to verify expression patterns. (B) This picture shows a magnification of nucleic acid staining.

unequal internal diameters connected to a base, which can be adjusted with 2-μm screws. The recipient block is fixed on the ground plate. The needles can be positioned in exact locations by adjusting the micrometer screws.

The first step is to punch out tissue from the donor block with the larger diameter needle. After switching the wing that holds the needles, the recipient block is punched to create a space for the tissue cylinder with the slightly smaller diameter needle. The wing is turned again, and the donor tissue is pushed out of the needle into the prepared hole with a stylet (Figure 1). At this point, it is critical to place every core with the same length to the same depth in the recipient block. One challenging aspect of TMA construction is utilizing donor tissue blocks that have different thicknesses. The thickness of the block determines how many sections of the TMA will be contained.

Cell lines are frequently included in TMAs as controls (Scherf et al., 2000). In fact, arrays with entire cell lines (*e.g.*, the NCI60 cell line panel) have been constructed to test potential chemotherapeutic agents (Figure 2). Recently, cell line and xenograft TMAs have been integrated into the preclinical drug development environment.

Transfer of sections to slides

Arrays may be sectioned with regular charged slides or tape transfer slides. For sections of whole tissue blocks and arrays with a small number of cores, sections can be transferred using a warm water bath. The section is taken from the microtome and gently placed on the surface of the warm water bath to even wrinkles, and a glass slide is used to pick up the section from the water bath. The tissue adheres to the slide as it is allowed to dry, usually in an oven. For IHC, proper adhesion of the tissue to the slide is critical. Early approaches used by laboratories included coating the slide with albumen or proteoglycans. To save time and to obtain a consistent quality, commercial slides coated with organic polymers (*e.g.*, poly-L-lysine) are currently used (Rentrop et al., 1986; Sompuram et al., 2004).

In working with large arrays, it is critical to cut and transfer the sections to the slide properly. Frequently, the cores get lost or the grid twists out of shape. Many sections of an array are lost when they become twisted or folded during this process, significantly reducing the number of usable slides. As a result, many laboratories use a tape transfer system to maximize the number of slides produced from a recipient block. With the adhesive-coated slide system manufactured by Instrumedics (Hackensack, NJ), a special piece of tape is placed directly on the block within the microtome. After cutting, the section remains on the tape, unaltered. The tape with the section is then positioned on a slide coated with artificial resin (Figure 1). This compound is then exposed to ultraviolet light to harden the resin. After placing the slide in a solvent, the plastic tape is removed and the section is left on the slide for further processing.

The recipient blocks should be sectioned on the same microtome used for block preparation before arraying. Although section thickness can be adjusted to preference, in tape transfer systems, sections thinner than 5 μm frequently result in uneven thickness. In order to maximize the number of high-quality slides, the entire block should be cut at once, or at least in groups of 20 slides or more—rather than cutting it as needed.

Quality control

Hematoxylin and eosin staining is the simplest method to assess the quality of an array. It is important to determine core retention, as well as to verify the correct tissue in each core at different depths of the block. Typically, sections 1, 50, 100, etc, are examined. Following hematoxylin and eosin staining, the quality of the tissue in term of proteins and nu-

cleic acids may be assessed. IHC and ISH are established techniques. However, although IHC has been widely used for decades, there is still a high intra- and inter-laboratory variability in the results, primarily due to inter-laboratory differences in antigen retrieval, staining protocols, antibodies used, and the interpretation of staining results. Therefore, approaches to guarantee a high level of quality may be highly variable. Vimentin is a common marker used to test immunoreactivity. This works well for whole sections; however, the core size is too small to ensure that vimentin-positive cells will be present in the core. Recently, many investigators have used CD31, but this vascular marker may not detect problems with particular oxidation-labile markers, which require specialized testing (Fergenbaum et al., 2004). For the integrity of nucleic acids, typically ISH with a housekeeping gene, such as actin, GAPDH, histone genes, or a ribosomal gene (*e.g.*, U6), is used (Geiszt et al., 2003). The quality of IHC procedures may be improved by the consistent use of positive and negative control sections for all IHC incubations.

Data analysis

Data collection and analysis

The greatest challenge associated with TMA technology is the collection and analysis of the data, which is difficult compared with other array platforms given the complexity of tissues and staining patterns. Despite the availability of automated image analysis systems, manual inspection and verification of the data at some level is still required. Manual scoring systems are either qualitative or semiquantitative, but none offer true continuous quantitative data. Qualitative scales are either binary (+ or -) or normative (0, 1, 2, 3, 4), typically providing categorization of a feature, such as intensity. Simple qualitative scores (*e.g.*, percent positive cells) that are limited in scope are frequently applied to nuclear staining (Hendriks et al., 2003; Nishizuka et al., 2003; Wang et al., 2002). The manual method also requires the interpretative skills of well-trained investigators, including pathologists. Pathologists frequently attempt to qualify patterns rather than strictly assigning them to a group. Importantly, this practice should be roundly discouraged, as it weakens the analysis that can be made. Many investigators will choose to replicate scoring systems that have previously been published, whereas others will use a similar method and scale for as many stains as they can. It is impossible to determine *a priori* what the optimal scoring system for a particular stain will be; therefore, flexibility in this capacity is essential. Importantly, staining patterns used in clinical practice should be scorable as positive or negative whenever possible. Any added complexity in a scoring system reduces its inter- and intra-observer reproducibility.

With the ability to process large amounts of quantitative data quickly, automated image analysis systems are increasingly being used with TMA technology. However, all of the pitfalls of data interpretation remain, and the analysis software is only as effective as the user who has programmed its functionality.

The first step in automated image analysis and scoring is the capture of a high-resolution image. High-resolution, optimally focused images are absolutely necessary for sufficient image analysis. Low-resolution or out-of-focus images show "bleeding" effects. Theoretically, it is not difficult to quantify the intensity and area of brown or blue structures using image analysis or editing (*e.g.*, Photoshop) software (Lehr et al., 1999). In practice, however, every slide is stained differently, depending on the laboratory performing the staining, the procedures used, and other parameters that vary from day to day. Any image analysis program used should offer manual adjustment of the staining conditions, thresholds, saturation, contrast, and other parameters of individual slides.

Fluorescent staining systems offer several advantages, including a greater dynamic range, a spectrum of colors, and simultaneous double staining for the control and target antigen (Camp et al., 2002; Haedicke et al., 2003; Rao et al., 2002). Fluorescent-stained TMAs can be also be scanned with a cDNA microarray scanner at high resolution (Haedicke et al., 2003). Unfortunately, this method provides only a "50,000 foot" view, with a lack of cellular detail. In addition, all fluorescent methods are limited by the loss of histomorphology, which can be essential in the correct interpretation of some staining patterns.

Managing the data

If the goal is a simple target-verification schema, the data generated with a TMA can be managed easily with a spreadsheet program (Manley et al., 2001). However, studies are now applying multiple stains to a single TMA or a series of TMAs, generating large, complex datasets. Data management issues generally become problematic for those users who wish to maintain images of the cores within the databases. Although a number of different solutions have been developed to address this dilemma, including a TMA data exchange platform that allows users to share results and images (Berman et al., 2003), none has been widely adopted.

Statistics analysis

The final data are typically exported into biostatistical analysis programs, such as SAS (Cary, NC) or SPSS (Chicago, IL). Correlation calculations, *t*-tests, or regression analyses may be performed, as may multivariate analysis using Cox proportional hazard models (Khanna et al., 2002; Poremba et al., 2002). End-point or epidemiological studies with Kaplan-Meier curves are often conducted to demonstrate the survival time corresponding to a certain protein expression level. Some investigators have attempted to use unsupervised cluster analysis and dendrogram approaches in the analysis of TMA data. This approach is severely flawed and must be avoided, as the choice of stains or probes applied is not unsupervised. However, the use of principle component analysis may be useful in visualizing the data.

Application of TMA technology

Basic research

TMA technology is used in basic research applications, such as the verification of gene expression patterns. Although investigators may examine mRNA levels with ISH (Erez et al., 2004; Geiszt et al., 2003), the current trend is to evaluate the function and expression of encoded proteins with IHC. TMAs offer well-defined, known tissue samples, which are chosen by a pathologist. However, this does not mean that there is no role for the pathologist in reviewing slides after the experiment has been performed; indeed, tissue arrays still offer all the complexity of diverse structures consisting of different cell types, which requires visualization and interpretation by appropriately trained professionals (Hewitt, 2004). Preselecting and presenting the tissue using a concise, high-throughput platform eases the chore of interpretation. With TMA technology, investigators can confirm tissue and protein expression and accurately compare expression levels and the frequency of expression in large panels of tissues.

Translational research

TMAs are increasingly being used in clinical protocols and have been successful in discriminating differences in outcomes not achievable through analysis of whole sections (Simon et al., 2001). It is essential to have basic knowledge of the expression pattern of a targeted protein or pathway. A TMA is a platform that can be used to "interrogate" tissue biomarkers in a systematic fashion. These tissue biomarkers can be protein, RNA, or DNA alterations that are correlated with a disease process. In clinical research, there are two areas of concentration in biomarker development. In drug development, the verification of candidate biomarkers is a challenging issue. Investigators assess the prevalence of a particular target or interrogate the functional status of a particular pathway. On the diagnostic side, biomarkers predict the outcome or response to therapy. Although the targets studied in both areas of biomarker development may be the same, the goals are different. For diagnostics and prognostication, the goal is to make the assay useful clinically. Developing new biomarkers that improve on current standards of care is challenging, as a biomarker must be faster, cheaper, and of greater utility before it can be adopted for clinical use.

Investigators are no longer only examining the expression of a single protein; instead, they are looking at multiple proteins within a signaling pathway and interrogating the activated form of the protein via antibodies specific to phosphorylated forms of the protein. These efforts are complicated, as they may require multiple antibodies against a single protein to determine the significance of the different sites of phosphorylation. Therefore, it is essential to use a scoring system with automated quantification for complete analysis.

Since TMAs can be constructed from archival paraffin-embedded tissue, they open up the vast archive of patient samples and make them accessible for medical research. TMAs play an ever-increasing role in translational medicine, bridging the chasm of discovery from the research bench to patient care.

Blood vessels and shear stress

TMA technology has been used to investigate the cellular mechanisms of vascular physiology and pathophysiology and, specifically, the expression profile of endothelial cells and vascular smooth muscle cells exposed to shear stress (De Nigris et al., 2001; Ohura et al., 2003). Similarly, the technology has demonstrated that mechanical strains, such as static pressure, induce changes in connective tissue growth factor expression in cultured human mesangial cells (Hishikawa et al., 2001). Many genes are differentially expressed in flow-loaded compared with normal flow tissue, including heme oxygenase 1 (HO-1). Increased HO-1 expression was confirmed by reverse transcriptase polymerase chain reaction, and IHC localized HO-1 expression to infiltrative macrophages (Nakahashi et al., 2002). These studies using histochemical/immunohistochemical staining and *in situ* hybridization have led to the identification of molecules that participate in shear-stress related vascular dysfunction.

Vascular dysfunction, atherogenesis, and neovascularization

Atherosclerosis is a multifactorial, temporally dynamic disease that can be modulated by a large number of environmental and genetic factors (Palinski and Napoli, 2002; Ross, 1999). TMA technology is suitable for sifting through the spectrum of candidate genes in order to focus specifically on those associated with atherogenesis (Fleiner et al., 2004). For example, human monocytes/macrophages respond to mechanical deformation with the selective augmentation of matrix metalloproteinases and the induction of immediate-early genes. In human monocytes/macrophages and THP-1 cells, biomechanical strain can induce the expression of the class A scavenger receptor, an important lipoprotein receptor in atherogenesis. In addition, cyclic mechanical strain induces only a few genes, including interleukin-8 and IEX-1 in THP-1 cells. Therefore, biomechanical deformation of monocytes/macrophages contributes to the degradation of the extracellular matrix and monocyte differentiation, and promotes atherosclerosis (Yamamoto et al., 2003).

TMA technology has recently been used to study vascular function in aneurysms and dissections (Koullias et al., 2004; Koullias et al., 2004). The increased MMP expression in aortic aneurysms and dissections indicated a metamorphosis in the aneurysm wall toward increased proteolysis compared with the normal aorta. MMP-2 and MMP-9 were more prevalent in aortic dissections. In both aneurysms and dissections, this transformation to a proteolytic state likely plays an important pathophysiologic role in the development and progression of the aortic disease.

The study of the cerebral circulation has generated much interest due to unsolved therapeutic issues, such as stroke. Recent studies have attempted to understand the effects of hormonal replacement therapy on the cerebrovascular circulation thorough analysis of the distribution, localization, expression, quantification, and characterization of hormonal receptor subtypes, as well as changes in structural morphology in diseased and healthy cerebrovascular tissue (Harrod et al., 2005). Therefore, the application of new molecular biological techniques like TMA analysis may help us to find causal pathogenic mechanisms involved in stroke and cerebrovascular dysfunction.

Heart failure

Cardiac hypertrophy, vascular remodeling, and the progression to heart failure remain some of the most formidable issues in public health. Little is known about the pathophysiology of cardiac hypertrophy and dilated cardiomyopathy, which are directly involved in heart failure. TMAs promise to provide new understanding of the mechanisms behind cardiac hypertrophy, heart failure, and vascular disease. As the availability of this technology expands and the costs decrease, TMAs will be an essential means of identifying novel therapeutic and diagnostic targets in cardiovascular disease. In addition to identifying new targets, microarrays have the potential to decrease the long research and development time required to bring a concept from the bench to the bedside (Lee, 2000).

TMA analysis has been used to evaluate altered mRNA and protein expression necessary for cardiomyocyte apoptosis in heart failure (Kaab et al., 2004; Steenbergen et al., 2003). Kaab *et al.* (2004) performed pair-wise comparisons of gene expression in the following categories: atria versus ventricles, disease-regulated genes in the atria, and disease-regulated genes in the ventricles. In the 51 heart samples examined, 549 genes showed divergent distributions between the atria and ventricles (272 genes with greater expression in the atria; 277 genes with greater expression in the ventricles). Steenbergen *et al.* (2003) observed altered gene expression consistent with a pro-apoptotic shift in the TNF-α signaling pathway, as well as decreased expression of TNF-α- and NF-κδ-induced anti-apoptotic genes, such as growth arrest and DNA damage-inducible 45β, Flice inhibitory protein, and TNF-induced protein 3. Consistent with a role for apoptosis in heart failure, phosphorylation of BAD at Ser-112a was found to be significantly decreased.

Despite similar clinical end points, heart failure resulting from dilated cardiomyopathy or hypertrophic cardiomyopathy appears to develop through different remodeling and molecular pathways. Indeed, Hwang *et al.* (2002) found several genes differentially expressed between dilated cardiomyopathy and hypertrophic cardiomyopathy (*e.g.*, αB-crystallin, antagonizer of myc transcriptional activity, β-dystrobrevin, calsequestrin, lipocortin, and lumican). Haase *et al.* (2002) identified ventricular myosin light chain type 2, skeletal α-actin, long-chain-acyl-CoA-synthetase, and mRNA for the protein KIAA0465 as differentially up-regulated genes in dilated cardiomyopathy. Microarray technology provides us with a genomic approach for exploring genetic markers and the molecular mechanisms leading to heart failure.

Future direction

The TMA has become a powerful tool in high-throughput biology. Although frequently used in a supporting role to confirm the results of other methodologies, TMAs can also serve as a high-throughput platform in proteomics, as well as an in-

vestigative tool in pathology and translational medicine. However, TMAs are rarely quantitative at the level of protein expression. Analysis of multiple targets requires multiple slides, on which the tissue of interest is constantly changing as the donor block is sectioned. To overcome this disadvantage of TMA technology, we have developed a novel method of analyzing proteins by transferring them from a paraffin-embedded section of tissue to a stack of specialized membranes (Tangrea et al., 2003), which are then immunoblotted (Figure 1D) (Shung et al., 2006). This method converts a traditional tissue section into a multiplex platform for expression profiling. A single tissue section can be transferred to ten membranes, each of which is probed with different antibodies, detected with fluorescent secondary antibodies, and quantified using a microarray scanner. In this manner, the TMA is used as a reverse-phase protein array and opens the archive of paraffin-embedded tissue to new avenues of proteomics.

Conclusion

Although a relatively new technology, the TMA is rapidly evolving as an important tool in basic science research, especially in light of continued advances in imaging and image analysis techniques. TMAs that use archival surgical specimens will confer tremendous clinical benefit, as they represent a crucial link for medical research and will bring discoveries from the bench to the bedside. In the future, clinicians will be able to use the results obtain with TMA technology to assist in the diagnosis, prognosis, and development of novel therapies in cardiovascular disease.

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