Tissue Microarray

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INTRODUCTION

The tissue microarray (TMA) technique has been in use for 15 years. The technology was first described in 1987, but its use took off 11 years later, when Kononen and colleagues developed a device that could rapidly and reproducibly produce TMAs (Kononen *et al.*, 1998). This powerful, high-throughput technique can be used to assay hundreds of patient tissues arrayed on a single microscope slide.

THE TECHNIQUE

Following fixation, biopsies and excised tissue samples are usually embedded in paraffin blocks to facilitate their cutting on a microtome; this results in 5-μm tissue slides that can be stained with hematoxylin and eosin (H&E) and viewed under a microscope. The paraffin blocks can also be used as source of material from which to construct a TMA. In this procedure, areas of interest are marked on the H&E slides by a pathologist. Then, cylindrical tissue core biopsy specimens (Figure 1a) from the original formalin-fixed paraffin-embedded (FFPE) tissue donor blocks are punched out of the paraffin block using specialized TMA equipment and placed in a predrilled hole in a (new) recipient paraffin block at defined array coordinates (Jawhar, 2009; Camp *et al.*, 2008). Sectioning of this recipient paraffin block will reveal a slide with numerous small, round tissue sections through the cores punched out of the original blocks; hence, each original tissue sample is represented by one or more small "histospots" with a preset fixed diameter ranging from 0.6 to 2 mm (Figure 1b). The number of spots on a single slide depends on the core size, ranging from 40 to 800 spots (Camp *et al.*, 2008). Cores are placed at specifically assigned coordinates, which typically are recorded in a spreadsheet. To facilitate "reading" of the TMA slide, different known tissue cores (e.g., liver, thyroid) are placed at the outer margins and empty holes are left at predefined places. After the TMA has been constructed, the recipient block is heated to 37 °C to fix the cores; then, using a microtome, sections are cut from the TMA blocks to generate TMA slides for analysis (Jawhar, 2009).

There are variations on the "normal" TMA procedure. The cutting-edge matrix assembly array is produced by cutting and stacking sections in a serial manner to produce arrays that represent hundreds of specimens. Some researchers have made a TMA using frozen tissue; others have used cell lines and needle biopsies (Camp *et al.*, 2008).

ADVANTAGES

- Allows high-throughput analysis of multiple specimens at the same time.
- Allows semiquantitative scoring of immunohistochemical or hybridization signals.
- Allows use of minimal quantities of tissue.

LIMITATIONS

- Cores may not be representative of the whole tumor owing to tumor heterogeneity; in a heterogeneous tumor, multiple cores must be taken.
- Requires experienced personnel and expensive equipment.

APPLICATIONS

The increased use of new molecular biology techniques has revolutionized investigation of the pathogenesis and progression of diseases such as cancer. New markers, identified via molecular research in cellular and animal models, require clinical validation on histopathological human specimens. This translation from basic to clinical research is facilitated by the TMA technology, which enables investigators to screen for the expression of a specific protein on tissue samples from a large cohort of patients by immunohistochemistry or the presence of nucleotide sequences by *in situ* hybridization.

All applications currently performed on standard histological sections from FFPE tissue are possible using TMA. In contrast to a series of whole FFPE-tissue sections, stained separately via immunohistochemistry, the use of TMA slides allows semiquantitative scoring of the intensity of staining because all tissue samples on a TMA slide, including the controls, have been exposed to the same amount of primary and secondary antibody and chromogen. For example, Zhou *et al.* (2013) stained a TMA composed of melanoma samples from 169 patients to evaluate expression of fibroblast growth factor–inducible protein 14 (Fn14) and applied semiquantitative scoring (Figure 2) that enabled them to identify Fn14 as prognostic marker and therapeutic target in melanoma.

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Detection of specific gene sequences using fluorescence *in situ* hybridization (FISH) of biopsy specimens from a large patient cohort (Chen and Chen, 2013) is another application of TMA. FISH can be used to detect the presence or absence of specific gene sequences as well as their location; this technique has been found useful in the differential diagnosis of ambiguous melanocytic lesions (Chen and Chen, 2013).

ADVANTAGES

Simultaneous analysis of a large number of specimens: TMA provides high-throughput data acquisition. For example, if 100 consecutive sections from a TMA block containing 200 cores are used, 20,000 data points are obtained. In addition, the original blocks from which the cores were taken are conserved; a similar approach using the original block would require an enormous amount of labor and time—and, more importantly, consume the original paraffin block.

Construction of *ex vivo* tumor progression model: studying morphological and molecular changes through the stages of tumor progression is easily performed by constructing a TMA because it is possible to include different tumor progression stages. Figure 1a represents a TMA of squamous cell carcinoma (SCC) progression for which cores were taken from normal skin, *in situ* SCC, and invasive SCC of the same patient.

Experimental uniformity: in a TMA, each tissue core is treated in an identical manner (same antigen retrieval, same temperature, same incubation time, same washing procedure, and same reagent concentration); therefore, samples can be compared and the immunohistochemical results can be read out in a semiquantitative way. The alternative of assessing the level of staining in separate histological whole sections is difficult because of subtle differences in intensity. When the cores are taken from pathologist-identified regions of interest, immunohistochemical staining can often be scored reliably by individuals with only rudimentary training or the scoring can be done by an automated reader (Camp *et al.*, 2008).

Decreased assay volume, time, and cost: only a small amount of each reagent is needed to assay all cores at the same time, compared with separately assaying standard histologic whole sections from each donor.

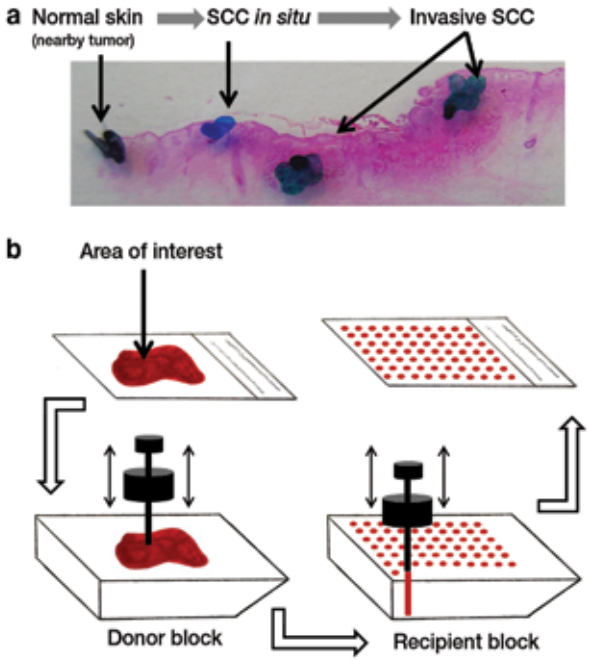

Figure 1. Construction of a TMA. (**a**) Example of marked areas of interest on a hematoxylin and eosin slide indicating sites at which to punch out samples of normal skin and *in situ* and invasive squamous cell carcinoma (SCC). (**b**) Process of constructing a TMA. The area of interest is marked on a hematoxylin and eosin slide. The TMA core is taken out of the original donor block and arrayed in a recipient block using a TMA needle. Multiple sections can be cut from the recipient block for morphological and molecular studies. TMA, tissue microarray.

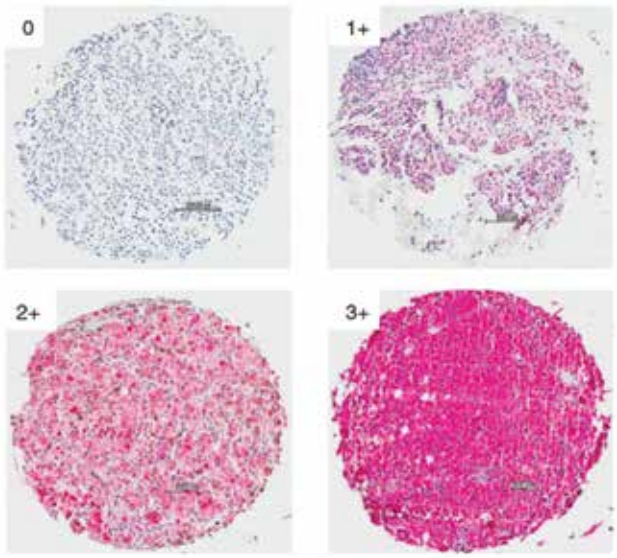

Figure 2. Scoring of a TMA. Semiquantitative scoring of a TMA immunostained with an anti-Fn14 antibody containing melanoma tissue. Reprinted with permission from Zhou *et al.*, 2013. TMA, tissue microarray.

DISADVANTAGES

Tissue heterogeneity: one of the most common criticisms of TMA is that the small cores may not be representative of the entire tumor, owing to tumor heterogeneity. This heterogeneity differs from cancer to cancer; for example, it has been estimated at 71% for SCC compared with 50% for breast cancer (Li *et al.*, 2010). The problem of tumor heterogeneity and consequent false-positive or false-negative results in the TMA slide can be overcome by including enough tissue cores per sample. Ideally, this number should be based on careful consideration before constructing the TMA, but in practice cores should be taken from all histologically divergent areas in the original sample, as divergent histology may result in divergent phenotype or genotype. In a previous study we took three or four cores from each vertical-growth-phase melanoma and obtained good concordance between immunohistochemical and reverse transcriptase–PCR data (Winnepenninckx *et al.*, 2006). Moreover, Jensen *et al.* (2011) obtained up to 96% agreement between TMA and whole-slide immunohistochemical data. Finally, the statistical power of analysis of hundreds of cases will largely eliminate the effect of variability of a single data point.

High cost: commercial TMA builder machines such as automated and semiautomatic tissue arrayers are expensive. A relatively simple and inexpensive alternative is the use of lab-made recipient paraffin blocks and ordinary cannula-piercing needles, skin biopsy punches, and bone marrow biopsy needles (Choi *et al.*, 2012). However this alternative is time-consuming, and for large sample investigations, automated or semiautomatic arrayers may ultimately be less expensive.

Variations in antigenicity: given TMA's ability to array many samples and to perform retrospective studies, variations in the antigenicity of stored archival samples may create problems.

COMPARISON WITH DNA MICROARRAYS

Every cell in the body contains a complete set of identical DNA; however, as a result of genetic and epigenetic mechanisms, only certain genes are active in a given cell, differentiating that cell from others (Villasenor-Park and Ortega-Loayza, 2013). The active genes are transcribed into messenger RNA (mRNA), which is subsequently translated into proteins. These proteins are responsible for the behavior and function of the cell (Villasenor-Park and Ortega-Loayza, 2013). In contrast to cDNA microarrays, which focus on gene expression at the mRNA level but do not yield information on the final steps of translation to a protein, TMAs can provide information on the expression level and activity of the final product, the protein.

CONFLICT OF INTEREST

The authors state no conflict of interest

CME ACCREDITATION

This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of the Duke University School of Medicine and Society for Investigative Dermatology. The Duke University School of Medicine is accredited by the ACCME to provide continuing medical education for physicians. To participate in the CME activity, follow the link provided. Physicians should only claim credit commensurate with the extent of their participation in the activity.

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SUPPLEMENTARY MATERIAL

A PowerPoint slide presentation appropriate for teaching purposes is available at http://dx.doi.org/10.1038/jid.2014.277.

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QUESTIONS

This article has been approved for 1 hour of Category 1 CME credit. To take the quiz, with or without CME credit, follow the link under the "CME ACCREDITATION" heading.

1. In constructing a TMA, the starting material most often used is:

A. Frozen tissue.
B. Needle-biopsy specimens.
C. FFPE tissue.
D. A cell line.

2. TMA is a powerful, high-throughput technique owing to its ability to assay hundreds of patient tissues arrayed on a single microscope slide for:

A. DNA crosslinking.
B. mRNA expression.
C. Invasion capacity of a single cell.
D. Protein expression.

3. Which statement does *not* apply to TMA?

A. More than 150 samples can be studied at the same time.
B. Only a small amount of reagent is needed to stain the samples.
C. TMA is an excellent tool for examining heterogeneous tissue.
D. TMA allows semiquantitative analysis of immunohistochemical signals.

4. Which statement is false?

A. A TMA can be made of frozen tissue.
B. Different tumor-progression stadia can be studied from the same patient.
C. Once a core is punched out from the original paraffin block, you cannot use it again.
D. The antigenicity of the different samples is always the same, even that of stored archival samples.

5. Which of the following is *not* an advantage of TMA?

A. Experimental uniformity.
B. Simultaneous analysis of a large number of specimens.
C. Decreased assay volume, time, and cost as compared with assaying whole sections.
D. Results can be obtained in less than one day.

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