Tissue Microarrays and Their Relevance to the Urologist

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Purpose: We review important aspects of TMA methodology and discuss its wide range of clinical applications with particular emphasis on key clinical studies. We also provide an update on recent and projected uses of this technology to help the urologist improve care in oncology patients.

Materials and Methods: A directed MEDLINE literature review of TMAs was performed. Important publications that have shaped our understanding of TMAs were selected for review. They were augmented by manual searches and our personal bibliographic collections.

Results: The TMA is a high throughput molecular biology technique that can significantly accelerate the processing of a large number of tissue specimens with excellent quality, good reliability and the preservation of original tissue. TMA studies demonstrate their accuracy and reliability compared to those of standard histological techniques and correlate with clinicopathological information to determine disease progression and prediction of the clinical outcome.

Conclusions: This review represents an overview and update for the urologist on TMAs and their clinical applications in urological oncology. In the future it is anticipated that the outcomes of this method will be used to assist in the diagnosis, prognosis and development of novel therapies in individual patients.

Key Words: tissue microarrays, urological oncology, prostate neoplasms, bladder neoplasms, renal neoplasms

The Human Genome Project[1–3] and recent advances in molecular biology techniques[4] have facilitated a better understanding of the pathogenesis of many diseases, such as cancer. In addition, potential molecular targets for the development of novel therapies have been identified.[5] In the field of urological oncology current knowledge of prostate, renal and bladder cancers has been dramatically influenced by these recent advances. A significant number of novel markers with diagnostic, prognostic and therapeutic significance in individual patients have been identified, for example with the use of DNA microarrays and proteomic analysis.[5–9] However, many of these studies used fresh tissue that was snap frozen and, therefore, the lack of long-term followup data has resulted in caution with regard to a meaningful outcome.

The accepted method of clinical validation of novel markers is on formalin fixed and paraffin embedded specimens using immunohistochemistry. Although this standard histopathological method provides extremely accurate, representative and reliable outcomes that can be reproduced effectively, it is extremely time-consuming, labor-intensive and costly if multiple markers require to be investigated on multiple tissue specimens. Furthermore, high throughput evaluation of clinical specimens results in the use of vital tissue resources because each histological block of tissue is typically exhausted after only 50 to 100 sections.

Thus, until recently the necessity of analyzing large numbers of human tissue specimens represented a major problem in translating the discoveries of novel markers at the gene level to clinical specimens and, therefore, to clinical practice. A decade after the innovative study of Battifora with his sausage block technique[10] the TMA was invented and developed by Kononen et al as a high throughput technique to assess thousands of different cores of pathological tissue simultaneously in a timely and cost efficient manner.[11] With this technique tissue from as many as 1,000 histology blocks can be placed at assigned locations on a newly created paraffin block using a custom-made machine. Multiple sections are then cut with the different tissue specimens maintaining their assigned locations. Subsequently morphological and molecular analyses using immunohistochemistry and in situ hybridization can be performed to determine the significance of multiple novel markers. It is now firmly established that the TMA can significantly accelerate the processing of a large number of tissue specimens with excellent quality, good reliability and the preservation of original tissue.

It is anticipated that urological practice will benefit from the use of TMAs in many aspects, including allowing rapid clinical validation of DNA microarray studies, particularly in urological cancer, providing the impetus for improvements in uropathology and allowing the identification of novel markers in frozen tissue and in cell lines as well as

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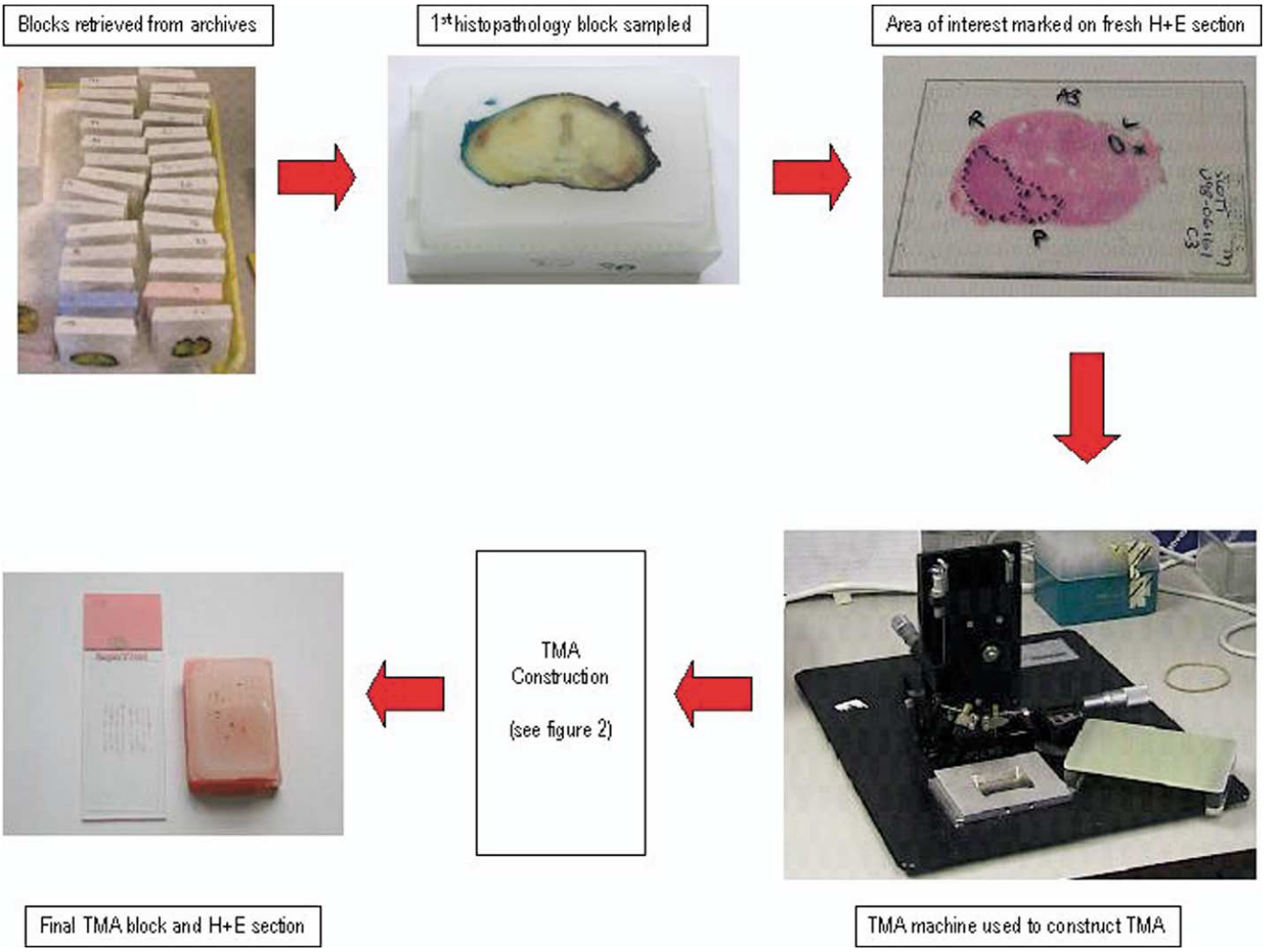

FIG. 1. Overview of TMA production

allowing the rapid identification of markers of diagnostic, prognostic and therapeutic significance (see Appendix).

METHODOLOGY

Figure 1 shows overall production of a TMA. Briefly, the initial step is to obtain the original histopathological blocks, that is donor blocks. A fresh section is cut onto a standard microscope slide and stained with hematoxylin and eosin. The area of study interest, commonly an area of cancer, is marked on the hematoxylin and eosin section. A tissue arraying instrument is used to acquire a tissue core from the donor block (fig. 2). Held in an X-Y precision guide containing a donor needle and a recipient needle on the TMA instrument this core is then placed in an empty paraffin block, that is the recipient block, at a specifically assigned location, which is accurately recorded, typically on a spreadsheet, such as Microsoft™ Excel. This sampling process is then repeated many times until hundreds or even thousands of cores are placed into the recipient block, producing the final TMA block. Following construction of the array block 200 to 300 sections can be cut with all cores in an identical configuration.

Currently 80% of TMAs are being used for immunohistochemistry, whereas the remaining 20% are being investigated by in situ hybridization techniques.[12] In addition, recent reports have been published on frozen tissue[13] and cell lines.[14] Subsequently using a simple computer program such as Microsoft™ Excel the locations of the cores on the TMA can be rapidly compared with clinical and pathological data.

ADVANTAGES OF TMAS

Compared with standard techniques the primary advantage of TMAs is the simultaneous analysis of a large number of specimens. As an example, if a TMA block containing 1,000 cores is cut 200 times, as many as 200,000 individual assays and, therefore, outcomes can be produced from a single block.[15,16] Other significant advantages over standard immunohistochemistry exist. Because all tissue specimens arrayed on 1 TMA are analyzed in identical fashion, antigen retrieval, reagent concentrations, incubation times with primary antibodies, temperatures and wash conditions are identical for each core, resulting in an unprecedented level of standardization over and above what is available using standard histopathological techniques.[17] In addition, since only