

BRIEF REVIEW

Tissue microarrays: a current medical research tool

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SUMMARY

Recent research in molecular biology has identified a significant number of novel markers, which may have diagnostic, prognostic and therapeutic significance. This is particularly pertinent in the field of cancer. Validation of these markers in multiple clinical specimens is currently performed by traditional histopathological techniques, which are disappointingly time consuming, labour intensive and, therefore, economically costly. These limitations have hampered the introduction of many novel markers into everyday clinical practice. The tissue microarray (TMA) is a high throughput technique, which allows the rapid and cost

effective validation of novel markers in multiple pathological tissue specimens. Tissue from up to a 1000 histology blocks can be arrayed accurately onto a newly created paraffin block, at designated locations. Subsequently, morphological and molecular investigations can be performed to determine the clinical significance of the novel markers tested. It is now firmly established that the TMA can significantly accelerate the processing of a very large number of tissue specimens with excellent quality, good reliability and preservation of original tissue, with ultimate clinical benefit.

Introduction

The investigation of the pathogenesis and progression of diseases such as cancer has been revolutionised with the increased use of new molecular biology techniques¹. Studies on clinical tissue have identified multiple novel markers, primarily at the gene level²⁻⁶. As the source of the tissue is invariably frozen, clinical follow-up data to correlate any meaningful outcome are lacking. In addition, reproducibility in everyday clinical practice is limited because of the expertise needed to perform these studies, and, more importantly, the cost implications. Current validation of markers on clinical

specimens is performed using standard histopathological techniques, which if multiple markers are investigated on multiple tissue specimens will prove to be time consuming, labour intensive and costly. The tissue microarray (TMA) is a high throughput molecular biology technique, which is anticipated to overcome these significant problems. For the purposes of this review, a systematic search of the literature was performed using the PubMed database (1966 to January 2004) with the search terms 'tissue microarrays', augmented by manual searches and the authors' personal bibliographic collections.

History of discovery

In 1986, Hector Battifora described a 'sausage' block method⁷, in which 1 mm thick 'rods' of tissue, obtained from different specimens, were wrapped carefully in a sheet of small intestine. This 'sausage' of tissues was embedded in a paraffin wax block, from which numerous sections were cut. Subsequently, this technique was modified from a 'sausage-block' to a 'checkerboard' configuration⁸. Although this technique conferred a significant advantage of simultaneously examining multiple tissue specimens under identical conditions, the inability to satisfactorily identify individual 'rods' limited any meaningful interpretation. These limitations were addressed subsequently, and in 1998, the TMA was first reported by Kononen *et al.*⁹.

Methodology

The stepwise production of a TMA (Figure 1) starts with obtaining the original histopathological blocks – the donor blocks. As these blocks are invariably stored in archives, it is recommended that a fresh section is cut onto a standard microscope slide and stained with Haematoxylin and Eosin (H&E). The area of study interest, commonly an area of cancer, is marked on the H+E section (Figure 2), in conjunction with an experienced histopathologist. The TMA is then ready to be arrayed. A TMA instrument, such as that from Beecher Instruments¹⁰, is used to acquire a tissue core from the donor block. This core is then placed in an

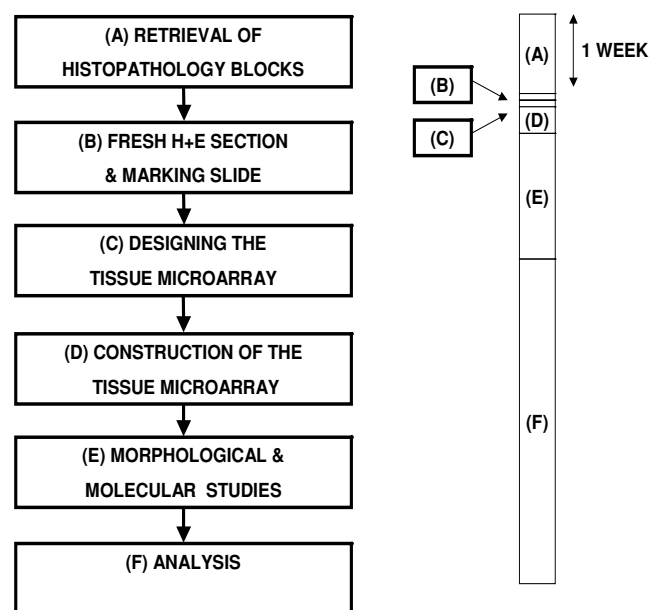


Figure 1. Stepwise production of tissue microarray (TMA)

empty paraffin block – the recipient block. As the instrument has a precision X–Y guide on it, the core can be placed at a specifically assigned coordinate, which is accurately recorded, typically on a spreadsheet, such as Microsoft Excel. The sampling process can then be repeated many times from different donor blocks until hundreds, or even thousands, of cores are placed into one recipient block, producing the final TMA block (Figure 2). Following construction of the array block, 200–300 sections can be cut, with all the cores in an identical configuration (Figure 2), in preparation for subsequent morphological or molecular analysis. Subsequently, using a simple computer programme, such as Microsoft Excel, the locations of the cores on the TMA can be rapidly compared with clinical and pathological data.

Morphological and molecular analysis of TMAs

At the present time, 80% of TMAs produced are analysed using immunohistochemistry, whereas most of the remaining TMAs are being investigated by *in situ* hybridisation techniques, such as interphase FISH¹¹. In addition, recent reports have been published on frozen tissue¹² and cell lines¹³.

Advantages of TMAs

The use of TMAs has significant advantages over standard techniques. TMAs allow the simultaneous analysis of very large numbers of specimens, allowing high throughput data acquisition. For example, if a TMA block containing 1000 cores is cut 200 times, as many as 200 000 individual assays, and therefore outcomes can be produced from a single block^{14,15}. Furthermore, as all the tissue specimens arrayed on one TMA are analysed in an identical fashion, antigen retrieval, reagent concentrations, incubation times with primary antibodies, temperatures and wash conditions are identical for each core¹⁶, resulting in an unprecedented level of standardization, over and above what is available using standard histopathological techniques. In addition, as only small quantities of reagent, and less laboratory personnel are required to perform the experiments, this method has proven to be extremely efficient and cost effective. The histopathological benefits include minimal destruction of the original tissue blocks, which are often considered as vital resources, the presence of internal positive and negative controls on the TMA, and

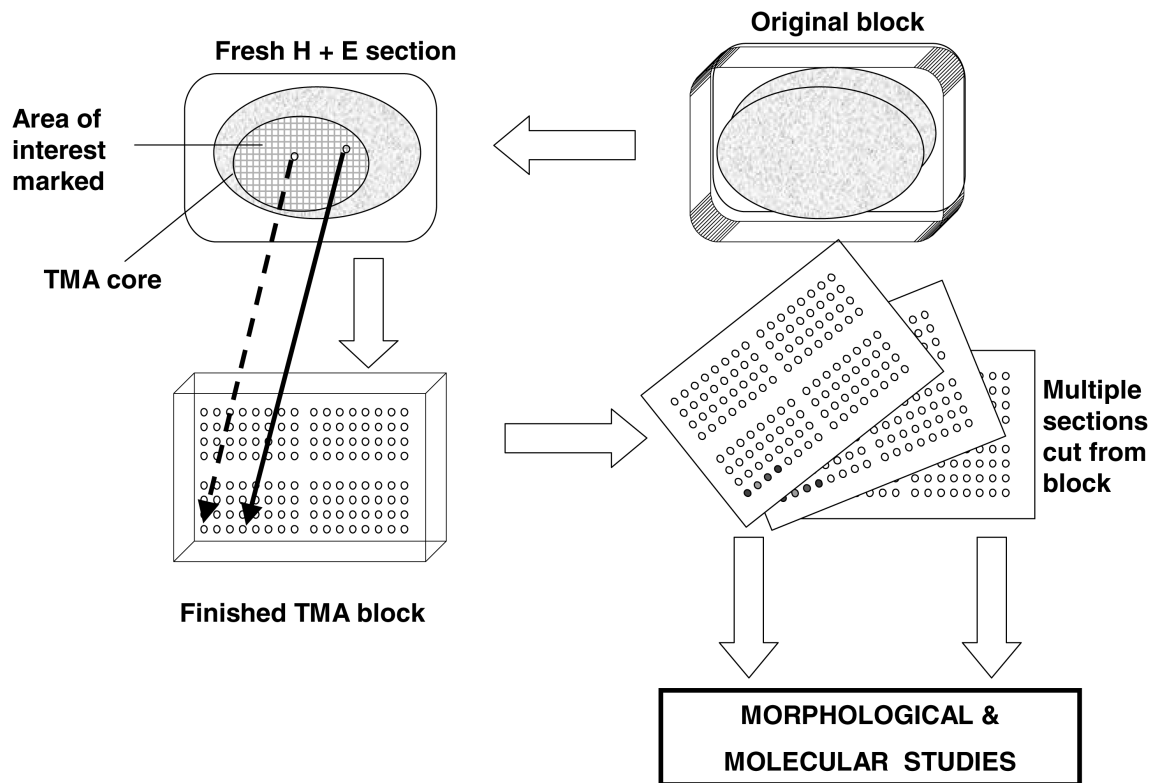


Figure 2. TMA construction

because such small specimens are sampled, many rare cell lines can be assessed with ease¹³.

arraying a larger number of arrays and lack of efficiency in processing of tissues.

Disadvantages of TMAs

One potential limitation of TMAs is that the small cores sampled may not be representative of whole tumours, particularly in heterogenous cancers such as prostate adenocarcinoma. This issue has been critically evaluated in studies where TMAs have been compared with whole mount sections. Excellent correlation has been reported between data obtained from the two techniques in a variety of tumour types such as breast¹⁷, prostate^{18,19}, bladder²⁰ and human fibroblastic tumours²¹. Furthermore, in the most extensive study investigating this issue of validation in prostate cancer Rubin *et al.*, found that 3–4 cores were the optimal number necessary to predict outcome after radical prostatectomy for localised prostate cancer¹⁸. Similarly, other groups have found that sampling with optimal cores was sufficient to accurately detect clinicopathological correlations^{19,21,22}. Interestingly, these studies have shown that increasing the number of cores, to compensate for heterogeneity and inevitable losses, only confers a slightly higher rate of validity, with the significant disadvantages of additional labour work in

Applications of TMAs

Clinical Validation of DNA Microarray Studies

Genes identified by DNA microarray studies require clinical validation on histopathological specimens for any meaningful outcome. TMAs are ideally suited for this purpose and most published studies using TMAs have reported on this application, allowing the identification of clinically relevant diagnostic and prognostic markers. Interestingly, the majority of these reports have been related to the analysis of tumour specimens. Depending on the type of tissue sampled and the clinical follow-up available, three categories of TMA can be defined.

(1) Multi-tumour Arrays

Many tumour types are sampled, from a diverse set of donor blocks, and arrayed on one recipient TMA block. With this type of TMA, a large group of tumours can then be expeditiously screened for the presence or absence of novel markers^{23–25}.

(2) Tumour Progression Arrays

Morphological and molecular changes through the different stages of tumour progression, of one particular tumour type, can be assessed in tumour progression TMAs. In prostate cancer, for example, construction of such an array would involve sampling of normal prostate, benign prostatic hyperplasia, prostatic intraepithelial neoplasia and different stages of prostate cancer, from localised disease to metastatic cancer. In one of the most significant papers on prostate cancer recently, a tumour progression array was used to show that the expression of a novel protein, *EZH2*, correlated with aggressiveness of disease²⁶. Similar studies have been published on breast cancer²⁷.

(3) Prognostic (Patient Outcome) Arrays

Whilst multi-tumour arrays and progression arrays provide useful diagnostic information, correlation of TMA derived data with clinical follow-up, to assess prognosis or patient outcome, is of significant interest to clinicians and their patients. One such example is the study involving the *EZH2* protein²⁶. In addition to its use as a marker of prostate cancer progression, Varambally *et al.*, showed that the degree of expression of this protein was related to outcome after radical prostatectomy. Whilst strong expression was associated with recurrence of tumour after surgery in a third of patients, weak *EZH2* staining was found in only 9% of individuals with clinical failure. Similar associations with prognosis have also been described in neoplasms of the breast²⁷, bladder²⁸ and kidney²⁹ using patient outcome arrays.

Recently, it has been suggested that combinations of expression of novel markers may be of more benefit in predicting prognosis³⁰. Rhodes *et al.* investigated 14 candidate prognostic markers in prostate cancer, including hepsin, AMACR, E-Cadherin and *EZH2*. They found that only the ratio of *EZH2* to E-Cadherin staining was statistically associated with prostate cancer recurrence following radical prostatectomy. This approach may be useful in identifying patients who are at high risk of recurrence and therefore providing them with alternative and appropriate treatment. As TMAs incorporate a high throughput approach, they are very well suited for this 'multiplex biomarker approach'.

Improvements in histopathology

It is known that there is significant inter-observer variation in the interpretation of tissue staining in

immunohistochemistry. TMAs have been shown to be an extremely useful tool for reducing this variation³¹ and therefore have a distinct advantage over standard section technique. Another simple, but effective and reliable method for internal quality control has been described with the use of internal control tissues in a 'mini-TMA format'³². In this technique the test tissues and the tissue of interest are stained under the same conditions with the identical concentration of antibody. The use of TMAs in routine immunohistochemistry and *in situ* hybridization techniques has been demonstrated for antibodies and probes currently available³³⁻³⁵. With the introduction of new markers, TMAs will be ideally suited for rapid and accurate optimisation³⁶, avoiding the need to extinguish vital archival histopathological specimens. Furthermore, it may be an extremely important aid for teaching histopathology trainees, and indeed clinicians who work in close conjunction with them. A recent publication indicated that 90% of pathologists considered the TMA approach useful for resident training and for pathology teaching³¹.

Analysing frozen assays and cell lines

A recently described use for TMAs includes the analysis of frozen tissue¹² and cell lines¹³. Although experimental at the present time, the TMAs can be stored and re-evaluated when a novel marker is subsequently identified. This will clearly have a major impact on translational research for the future by the rapid transfer of results from cell lines and animal models to human tumours.

Automation

Attempts have been made to automate the process of TMA construction. Various machines are now available which may array single and multiple TMA blocks in an even shorter period of time than is currently performed manually^{10,37}. Furthermore, major attempts are now in progress to automate the data analysis step. This process was initiated by acquiring and storing digital images of the TMA on a computer using technology such as BLISS (the Bacus Labs Incorporated Slide Scanner)^{38,39}. Furthermore, Camp *et al.*⁴⁰, have developed a system which uses fluorescent imaging to quantifying TMA cores at the sub-cellular level, to provide a score that is directly proportional to the number of molecules expressed per unit area. This is termed Automated quantitative analysis, or *AQUA*, and if further validated, may eventually eliminate the need for a pathologist to

interpret each TMA core. Software programs have also been developed to aid in the analysis of immunohistochemistry staining data⁴¹. One such program, called *Treeview*, allows for rapid transformation of immunohistochemistry data into a format that can be used for cluster analysis⁴². As can be observed in Figure 1, the majority of time taken to perform a TMA study is on data analysis. From this, one can conclude that automated construction will probably have little impact on speed of TMA construction, except in extremely large research units, whereas automated analysis will significantly reduce the overall time required to complete a study.

Tissue Array Research Program

The National Human Genome Research Institute (NHGRI) and the National Cancer Institute (NCI) have created the Tissue Array Research Program (TARP) in an attempt to promote research and development of TMAs⁴³. The overall aims of TARP are to produce TMAs for use by the research community, to provide assistance in arraying unique tissue materials, such as those from clinical trial groups, and to provide training, workshops and protocols regarding TMA technology.

Conclusion

Currently, the TMA is mainly used for the high throughput investigation of candidate genes, and their proteins, identified by molecular biology techniques such as DNA microarrays. Studies demonstrate their accuracy and reliability compared to standard histological techniques as well as exhibiting correlation with clinico-pathological information to determine progression of disease and prediction of outcome. In the future, it is anticipated that doctors in primary care, and hospital practice will be able to use the outcomes from this method to assist in the diagnosis, prognosis and development of novel therapies for individual patients, particularly in diseases such as cancer.

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