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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 techniques[1]. Studies on clinical tissue have identified multiple novel markers, primarily at the gene level[2–6]. 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 method[7], 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' configuration[8]. 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 Instruments[10], is used to acquire a tissue core from the donor block. This core is then placed in an 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.

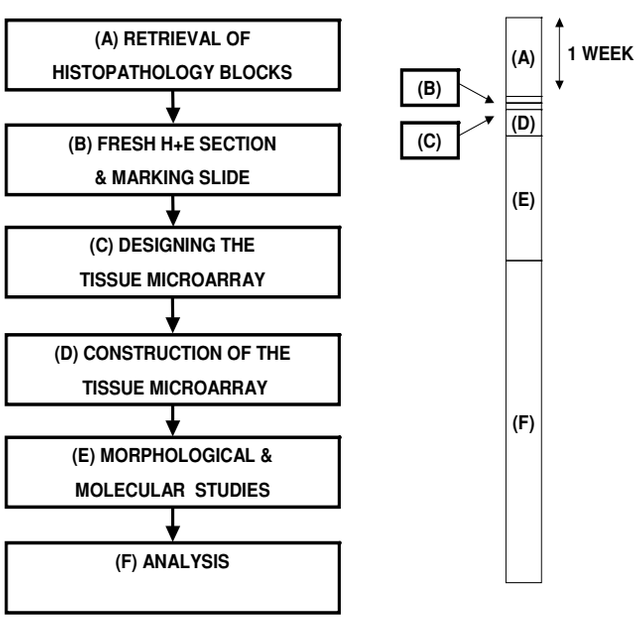

Figure 1. *Stepwise production of tissue microarray (TMA)*

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 FISH[11]. In addition, recent reports have been published on frozen tissue[12] and cell lines[13].

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 core[16], 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

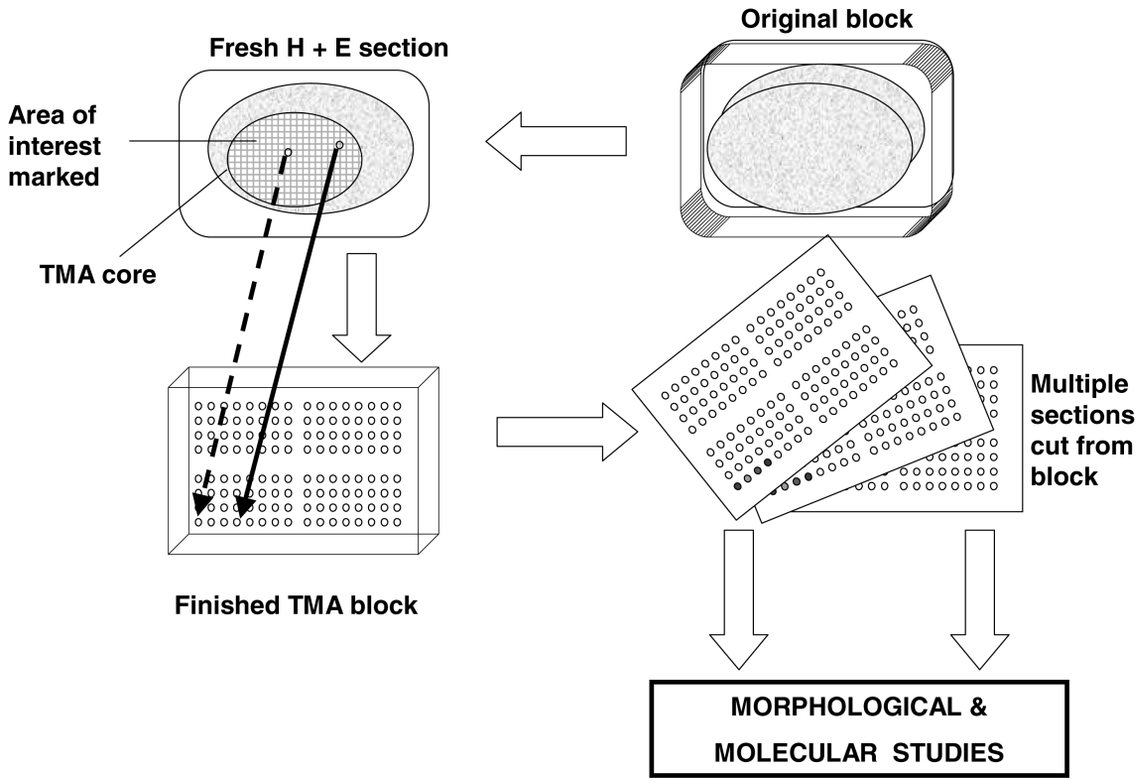

Figure 2. *TMA construction*

because such small specimens are sampled, many rare cell lines can be assessed with ease[13].

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 breast[17], prostate[18,19], bladder[20] and human fibroblastic tumours[21]. 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 cancer[18]. Similarly, other groups have found that sampling with optimal cores was sufficient to accurately detect clinico-pathological 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 arraying a larger number of arrays and lack of efficiency in processing of tissues.

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 disease[26]. Similar studies have been published on breast cancer[27].

(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 protein[26]. 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 breast[27], bladder[28] and kidney[29] using patient outcome arrays.

Recently, it has been suggested that combinations of expression of novel markers may be of more benefit in predicting prognosis[30]. 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 variation [31] 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 available[33–35]. With the introduction of new markers, TMAs will be ideally suited for rapid and accurate optimisation[36], 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 teaching[31].

Analysing frozen assays and cell lines

A recently described use for TMAs includes the analysis of frozen tissue[12] and cell lines[13]. 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 data[41]. One such program, called *Treeview*, allows for rapid transformation of immunohistochemistry data into a format that can be used for cluster analysis[42]. 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 TMAs[43]. 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.

References

1. Williamson M, Naaby-Hansen S, Masters JR. 21st century molecular biology in urology. BJU Int 2001;88:451-57
2. Dhanasekaran SM, Barrette TR, Ghosh D, et al. Delineation of prognostic biomarkers in prostate cancer. Nature 2001;412:822-26
3. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000;406(6797):747-52
4. Zou TT, Selaru FM, Xu Y, et al. Application of cDNA microarrays to generate a molecular taxonomy capable of distinguishing between colon cancer and normal colon. Oncogene 2002;21(31):4855-62
5. Takahashi M, Rhodes DR, Furge KA, et al. Gene expression profiling of clear cell renal cell carcinoma: gene identification and prognostic classification. Proc Natl Acad Sci USA 2001;98(17):9754-9
6. Sanchez-Carbayo M. Use of high-throughput DNA microarrays to identify biomarkers for bladder cancer. Clin Chem 2003;49:23-31
7. Battifora H. The multitumor (sausage) tissue block: novel method for immunohistochemical antibody testing. Lab Invest 1986;55:24-8
8. Battifora H, Mehta P. The checkerboard tissue block. An improved multitissue control block. Lab Invest 1990;63(5):722-4
9. Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998; 4:844-47
10. http://www.beecherinstruments.com [Accessed 1 February 2004]
11. http://www.pathology.pitt.edu/apiii02/apiii2002_SHewitt/S-Hewitt-APIII-02x.html [Accessed 1 February 2004]
12. Fejzo MS, Slamon DJ. Frozen tumor tissue microarray technology for analysis of tumor RNA, DNA, and proteins. Am J Pathol 2001;159(5):1645-50
13. Brandt B, Roetger A, Bidart JM, et al. Early placental insulin-like growth factor (pro-Epil) is overexpressed and secreted by c-erbB-2-positive cells with high invasion potential. Cancer Res 2002;62:1020-4.
14. Simon R, Struckmann K, Schraml P, et al. Amplification pattern of 12q13-q15 genes (MDM2, CDK4, GLI) in urinary tract bladder cancer. Oncogene 2002;21,2476-83
15. Mousses S, Kallioniemi A, Kauraniemi P, Elkahloun A, Kallioniemi OP. Clinical and functional target validation using tissue and cell microarrays. Curr Opin Chem Biol 2002;6:97-101
16. Rimm DL, Camp RL, Charette LA, Olsen DA, Provost E. Amplification of tissue by construction of tissue microarrays. Exp Mol Pathol 2001; 70:255-64
17. Zhang D, Salto-Tellez M, Putti TC, Do E, Koay ES. Reliability of tissue microarrays in detecting protein expression and gene amplification in breast cancer. Mod Pathol 2003;16(1):79-84
18. Rubin MA, Dunn R, Strawderman M, Pienta KJ. Tissue microarray sampling strategy for prostate cancer biomarker analysis. Am J Surg Pathol 2002;26:312-19
19. Mucci NR, Akdas G, Manely S, Rubin MA. Neuroendocrine expression in metastatic prostate cancer: evaluation of high throughput tissue microarrays to detect heterogeneous protein expression. Hum Pathol 2000;31(4):406-14
20. Nocito A, Bubendorf L, Maria Tinner E, et al. Microarrays of bladder cancer tissue are highly representative of proliferation index and histological grade. J Pathol 2001;194:349-57
21. Hoos A, Urist MJ, Stojadinovic A, et al. Validation of tissue microarrays for immunohistochemical profiling of cancer specimens using the example of human fibroblastic tumors. Am J Pathol 2001;158(4):1245-51
22. Camp RL, Charette LA, Rimm DL. Validation of tissue microarray technology in breast carcinoma. Lab Invest 2000;80:1943-9
23. Kallioniemi OP, Wagner U, Kononen J, Sauter G. Tissue microarray technology for high throughput molecular profiling of cancer. Hum Mol Genet 2001;10:657-62
24. Andersen CL, Monni O, Wagner U, et al. High-throughput copy number analysis of 17q23 in 3520 tissue specimens by fluorescence in situ hybridization to tissue microarrays. Am J Pathol 2002;161(1):73-9
25. Schraml P, Kononen J, Bubendorf L, et al. Tissue microarrays for gene amplification surveys in many different tumor types. Clin Cancer Res 1999;5:1966-75
26. Varambally S, Dhanasekaran SM, Zhou M, et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature 2002;419:624-29
27. Simon R, Nocito A, Hubscher T, et al. Patterns of her-2/neu amplification and overexpression in primary and metastatic breast cancer. J Natl Cancer Inst 2001;93(15):1141-6
28. Simon R, Struckmann K, Schraml P, et al. Amplification pattern of 12q13-q15 genes (MDM2, CDK4, GLI) in urinary bladder cancer. Oncogene 2002;21(16):2476-83
29. Moch H, Schraml P, Bubendorf L, et al. High-throughput tissue microarray analysis to evaluate genes uncovered by cDNA microarray screening in renal cell carcinoma. Am J Pathol 1999;154:981-6
30. Rhodes DR, Sanda MG, Otte AP, Chinnaiyan AM, Rubin MA. Multiplex biomarker approach for determining risk of prostate-specific antigen-defined recurrence of prostate cancer. J Natl Cancer Inst 2003;95:661-8
31. De la Taille A, Viellefond A, Berger N, et al. Evaluation of the interobserver reproducibility of Gleason grading of prostatic adenocarcinoma using tissue microarrays. Hum Pathol 2003;34(5):444-9

32. Packeisen J, Buerger H, Krech R, et al. Multi tissue arrays – a new approach for quality control in immunohistochemistry. J Clin Pathol 2002;55:613-5
33. Parker RL, Huntsman DG, Lesack DW, et al. Assessment of interlaboratory variation in the immunohistochemical determination of estrogen receptor status using a breast cancer tissue microarray. Am J Clin Pathol 2002;117:723-8
34. Hsu FD, Nielsen TO, Alkushi A, et al. Tissue microarrays are an effective quality assurance tool for diagnostic immunohistochemistry. Mod Pathol 2002;15:1374-80
35. Mengel M, von Wasielewski R, Wiese B, Rudiger T, Muller-Hermelink HK, Kreipe H. Inter-laboratory and inter-observer reproducibility of immunohistochemical assessment of the Ki-67 labelling index in a large multi-centre trial. J Pathol 2002;198:292-9
36. Packeisen J, Korsching E, Herbst H, Boecker W, Buerger H. Demystified...tissue microarray technology. Mol Pathol 2003;56(4):198-204
37. http://www.chemicon.com/company/PR/Arrayer.asp [Accessed 1 February 2004]
38. http://www.bacuslabs.com/WebSlideOverview.html [Accessed 1 February 2004]
39. Bova GS, Parmigiani G, Epstein JI, Wheeler T, Mucci NR, Rubin MA: Web-based tissue microarray image data analysis: initial validation testing through prostate cancer Gleason grading. Hum Pathol 2001;32:417-27
40. Camp RL, Chung GG, Rimm DL. Automated subcellular localization and quantification of protein expression in tissue microarrays. Nat Med 2002;8:1323-7
41. Liu CL, Prapong W, Natkunam Y, et al. Software tools for high-throughput analysis and archiving of immunohistochemistry staining data obtained with tissue microarrays. Am J Pathol 2002;161:1557-65
42. http://ccr.cancer.gov/tech_initiatives/tarp/ [Accessed 1 February 2004]

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