Review Article

HIGH THROUGHPUT MOLECULAR PROFILING OF TUMOUR SPECIMENS- TISSUE MICROARRAY: A BRIEF REVIEW

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ABSTRACT:
Recent advances in the field of human molecular genetics have revealed gene based disease mechanisms in many areas of medicine. The study of new prognostic and diagnostic markers in large numbers of clinical specimens is an important step in translating the new findings from basic science to clinical practice. Tissue microarray is a recent innovation which facilitates analysis and bridges the gap between candidate discovery and candidate testing. They are routinely used molecular epidemiology, drug development, and determine the prognostic, diagnostic and predictive value of new biomarkers. This technology should not be confused with DNA microarrays where each tiny spot represents a unique cloned cDNA or oligonucleotide. In tissue microarrays, the spots are larger and contain small histologic sections from unique tumors or tissues. The purpose of this article is to review this increasingly popular technology, focusing on several technical aspects of tissue microarray applications.
Key words: Immunohistochemistry, Molecular profiling, Tissue micro arrays.

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INTRODUCTION

The practice of pathology is currently undergoing significant change, in large part due to advances in analysis of DNA, RNA and protein in tissues. These advances have permitted improved biologic insights into many development, inflammatory, metabolic, infectious and neoplastic diseases.[1] However, the recent use of high throughput technical approaches to analyze molecular alterations occurring during cancer development (i.e. expression and CGH arrays, protein analyses) have produced enormous pools of preliminary data that needs, eventually, to be validated . In order to define their clinical significance, large scale and well characterized clinical specimens need to be processed and screened for multiple markers, but the use of conventional approaches is tedious and time consuming. The application of tissue microarray (TMA) platforms facilitates such analysis, indeed it is very useful tool to bridge the gap between candidate discovery and candidate testing. Slides derived from TMA blocks can be used to study gene amplification and protein overexpression by DNA & RNA in situ hybridization (ISH) or by immunohistochemistry (IHC) with tremendous savings on analysis time in addition to labor

and reagent costs. The routine use of this technology can accelerate studies seeking the association between molecular changes, clinical end points and validation of novel biomarkers as well as transition of basic research results to clinical applications.[2,3] This technique has potential to allow validation of new genes at a speed comparable to the rapid rate of gene discovery afforded by DNA microarray.[3] The purpose of this article is to review the technological aspects of TMA and its application in biomedical sciences.

DESIGN, CONSTRUCTION AND TECHNIQUE OF TMA

Battifora et al[4] in 1986 described the "multitumor (sausage) tissue block" which they further modified in 1990 as the "checkerboard tissue block". In this technique described for constructing multitumor sausage tissue block described by Battifora, one mm thick 'rods' of different tissues were wrapped in a sheet of small intestine which was then embedded in a paraffin blocks.[4,5] TMAs are composite paraffin blocks constructed by extracting cylindrical tissue core biopsies from different paraffin donor blocks and re-embedding these into single recipient block at defined array co-ordinates.[6,7] A tissue microarray instrument (for eg. Beecher instruments) is used to acquire a tissue core from the donor block.12. The current Beecher instruments arraying device is designed to produce sample circular spots that are 0.6mm in diameter at a spacing of 0.7-0.8 mm.[7] The surface area of each sample is 0.282mm^2 or in pathologists term, about the size of 2-3 high power fields. The use of spots on a single slide is variable depending on the array design; the current comfortable maximum with 0.6mm needle is about 600 spots per standard glass microscope slide.

The essential idea in arraying is to match the core size to the number of cores and put these cylinders of tissue friendly into the user friendly recipient block. The standard needles come in 0.6, 1.0, 1.5, 2.0 mm diameter. The maximum practical array area in the recipient block with each of these needles will be approximately 500, 200, 100, 50 cores respectively. A template grid with selected area size and individual sub arrays should be made prior to construction of tissue array. The choice of selecting a needle size is crucial depending on the quantity and quality of available tissue. A general dictum is that, the larger the sample size, smaller should be the core diameter. As the needle diameter increases, the spacing between cores must increase.[8] The core is placed at a specifically assigned co-ordinate (X-Y guide) which is accurately recorded, typically on a spreadsheet such as Microsoft Excel.[9]It is imperative that in each cycle a recipient hole is made first and then donor tissue is cored. If a series of recipient holes are made before hand, the holes will get deformed due to the elasticity of paraffin. It is always useful to make a pointer core on the top left of the array area of the recipient block for providing orientation. Normal controls should always be included in the array.[8]

TMA's are broadly classified into three types- Multi tumor array, Progression array (based on tumor stage) and prognostic arrays when tumor s with known clinical endpoints are arrayed. With multi tumor arrays, many tumor types are sampled from a diverse set of donor blocks, and arrayed on one recipient tissue microarray block. With this type of tissue microarray, a large group of tumors can then be expeditiously screened for the presence or absence of novel markers.[7,10,11] For tumor progression arrays, morphological and molecular changes through the different stages of tumor progression, of one particular tumor type, can be assessed in tumor progression tissue microarrays.[7] In prognostic (patient outcome) arrays, correlation of tissue microarray-derived data with clinical follow-up, to assess prognosis or patient outcome, is of significant interest to clinicians and their patients.[7,9]

ADVANTAGES AND LIMITATIONS OF TMAs

There are numerous advantages & limitations of TMAs over standard techniques like IHC, ISH and m RNA ISH on each tissue sample separately. These are illustrated in **Table 1**.

Table 1: Advantages and limitations of TMAs

ADVANTAGES	LIMITATIONS
Amplification of scarce resource: Instead of 50-100 conventional sections for analysis from one tissue biopsy, the micro array technique could produce material for 5,00,000 arrays represented as 0.6mm disks of tissue. Thus this technique essentially amplifies (up to 10,000 fold) the limited tissue resources.[7]	There is high variability in intralaboratory and interlaboratory results mainly due to inter laboratory differences in antigen retrieval, staining protocols, antibodies used and in interpretation of results.
For research purpose with decreased assay volume, time, cost: Researchers can study an entire cohort of cases simultaneously by staining just a few tissue microarray slides generating a large amount of data in a relatively shorter time.[7,9]	Small cores sampled may not l representative of the whole tumor, particular in heterogenous cancers such as prosta adenocarcinoma and hodgkin's lymphoma.[12]
Simulataneous analysis of very large number of specimens: TMA provides high throughput data acquisition and statistical significance of new markers can be precisely determined in a single experiment.[13,14]	A tumor tissue may comprise of mar different histologic areas within itself, such regions of apoptosis, necrosis or increase proliferation etc. and it may not be possible sample all areas in one tissue core.[15]
Mini TMA's with 16-25 cores can be used as internal controls when performing IHC, FISH and other tests to provide a high degree of intra laboratory reproducibility. Test tissue and tissue of interest are stained under identical conditions.[5,12]	Absense of one or more core sections on immunostained slides is another limitation.[16]
Original block for diagnosis is preserved and valuable tissue is conserved.	High cost of TMA facility limiting its use commonly in medical practice.
TMA's can be used for techniques such as immunologic stains with either chromogenic or fluorescent visualistion , fluorescence or mRNA insitu hybridization of histochemical stains. The variables such as temperature, incubation times, washing procedures and antibody and other costly reagents can be standardized for all the samples at the same time and less quantity is required to treat the entire cohort.[5]	Sample fixation and embedding has gre impact on quality of TMA sections. Buffere formalin can modify the RNA molecule b adding mono-methyl groups to its base whic has a damaging effect on RNA by alterir antigenic epitope structure, giving erroneo results of in situ analysis of DNA, RNA ar proteins.[17,18]
TMA's can provide possibilities for high throughput molecular profiling of clinical human tumors such as lung cancer, breast cancer, gliomas and prostate cancer.[5]	Loss of tissue cores during processing and unreliability of IHC staining.

ADVANCEMENTS IN TMA

The era of tissue microarrays has just begun. A multitude of different possibilities exist of which some are already in use. For e.g.

1. FROZEN TMA: Here TMA were constructed from unfixed frozen tissues and embedded in a recipient blocks made of optimal cutting temperature (OCT) media.[19] Although there is distortion of morphology in frozen TMAs and they are difficult to work on, they provide excellent target material for study of RNA, DNA and protein.[8]
2. CELL LINE MICROARRAYS (CMA): In stem cell science and particularly for induced pluripotent stem cells, CMA technology can offer several advantages particularly in screening cell population to search for bonafide clones.[2] Both suspension grown and adherent grown cells are placed in agarose gels, fixed and then embedded in paraffin. They are low cost, high throughput means of exploring proteins using cell lines.[20]
3. XENOGRAFT TUMOR ARRAYS (XMA): are used as preclinical model system in which assays are developed that measure either susceptibility or response to a drug.[8]

Furthermore digital techniques have taken center stage in clinical medicine as well as biomedical research. Recent pathology has begun to embrace this digital revolution.[7].The present TMA technology still has scope for its up gradation and more advanced versions of the current TMA technology will be required in future. Control over holing and sample depth, single use holing and sampling needles, quality of needles, design of arrays, sectioning and transferring techniques, staining and other molecular pathology techniques, less manual operation and more precise automation are some areas where TMA can be improved further. Association of Pathology Informatics (API)[5] Developed an open, community supported TMA data exchange specification for the first time.[21] This allows researchers to submit their data to journals and to public data repositories and to share or merge data different laboratories.

CONCLUSION

Tissue microarrays are used to confirm results from other experimental platforms, such as expression microarrays, as well as a primary tool to explore the expression profile of proteins by IHC analysis. Tissue microarrays are routinely used molecular epidemiology, drug development and determining the diagnostic, prognostic and predictive value of new biomarkers. By applying traditional protein based assays, as well as novel assays to the platform, tissue microarrays have gained a new utility as a proteomic tool for both basic science as well as clinical investigation.

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