REVEW ARTICLE

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Tissue microarrays, a revolution in pathology research

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ABSTRACT

A long-lasting problem in the analysis of tissue samples is the time-consuming and repetitive process of histologic preparation and pathologic review of tissue sections. These two critical factors, innate no nuniformity in preparation and subjectivity of analysis, therefore limit the scientific and statistical thoroughness of tissue based studies. Employing tissue microarray (TMA) technique provides a potential solution to each of these problems. This review discusses the methods of creating tissue microarrays, the advantages and disadvantages of the technology, analysis methods, and recent TMA applications.

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History of Tissue microarrays

The new idea of collecting different tissue samples together into one block was first established by Battifora in 1986.[1] Around one hundred various samples were cut into small pieces with a razor blade and then arranged for paraffin embedding into a multi-tumor "sausage" block.Wan et al modified this invention for precise localization of tissue samples with locating skin biopsy-type cores in fixed positions, resulting in the first true tissue microarray pictures.[2] Early applications of tissue arrays in breast cancer were performed by Press et al for study of Her2-neu expression and amplification in breast cancer.[3]

Kononen et al in 1998 published an updated version describing a new mechanical device to perform a precise tissue microarray construction for massive parallel high-throughput analysis by microarrays.[4]

This manual arraying device can punch paraffin blocks and insert the cores into a recipient array block. These tissue microarrays can contain up to 800 individual 0.6-mm cylindrical tissue cores in a single paraffin "recipient" block that can then be cut and analyzed. The sectioned slide can be applied for simultaneous processing and high-throughput analysis of DNA, RNA, and protein expression on hundreds of samples, representing hundreds of patients. In contrast to complementary DNA (cDNA) microarrays, in which hundreds or thousands of genes are evaluated from a single tissue sample or cell line, tissue microarrays allow the evaluation of a single gene (or gene product) on hundreds or thousands of tumors.

Tissue microarray construction

Array construction

The construction of tissue microarray generally begins with the collection of cases with tissue samples that have been fixed and paraffin embedded. For each tissue block, a section is cut and stained with a standard hematoxylin and eosin (H&E) stain. A pathologist reviews the H&E slide to identify and mark out the representative areas of tumor tissue to be included in the array (e.g., by circling the region of interest). The paraffin blocks ("donor" tissue) are then organized and arranged in the order in which they will be inserted into the "recipient" paraffin block. Arrays can be designed in many ways, usually including control spot and cores from different patients or multiple cores from the same patients.When tissue selection have been completed, the tissue samples can be arrayed onto a recipient block by using a Tissue Arrayer Minicore (ALPHELYS, Plaisir, France), or Beecher Instruments (Sun Prairie, WI) or Chemicon(Temecula, CA) manual arraying device.Tissue arrays can be constructed by placing 0.6 mm in diameter samples from different donor tissue and embedded into the recipient block, with 1.0 mm spacing separating each specimen. Although larger and smaller needles can be used, ranging from 0.2 mm to 2 mm. A maximum of approximately 800 cores could be placed in a standard-sized (0.282 mm²) single-recipient block. The overall steps illustrated in **Figure 1 and 2** .[5]

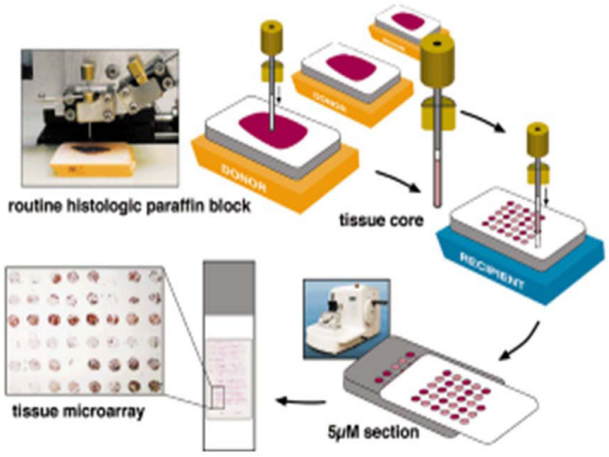

Figure 1: Overview of tissue microarray construction. Cores are removed from a set of paraffin-embedded "donor" blocks and inserted into a "recipient" block. The new block is then cut into 4-5-micron-thick sections. These sections can then be stained using standard laboratory methods.[5]

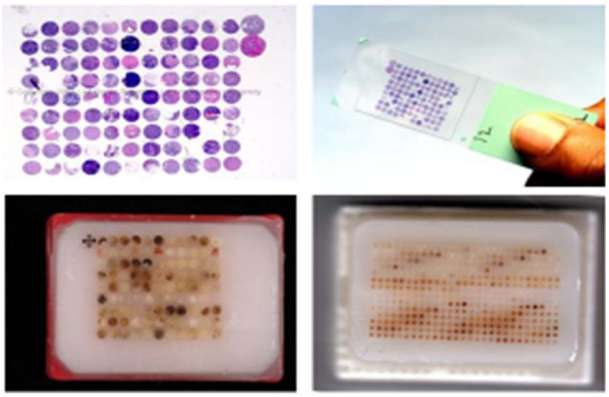

Figure 2: Example of slides and blocks of tissue microarray

Tissue Arrayer MiniCore

Tissue ArrayerMiniCore is built on an innovative and patented technology at ALPHELYS Company from France. It is a very compact instrument that allows rapid and accurate construction of low to high density tissue arrays. **(Figure3)**

Automated arrayer(Beecher instrument)

The ATA-27 automated arrayer is designed for making construction of high-density tissue microarray blocks fast, accurate and reliable. The instrument can accommodate nearly all current tissue cassettes and can be easily adapted to array large or odd-shaped archival specimens. The system equipped with high-performance PC preloaded with custom software for mapping donor cores to a single or multiple recipient cassettes. This system is particularly useful for creating arrays from very small tissue blocks and for making as many replicate arrays as possible **(Figure4)**.

Manual Tissue Arrayer (Beecher instrument)

Each manually operated tissue arrayer instrument included two pairs of punches with styles (0.6 mm diameter), one recipient block holder, one donor block bridge, operating manual containing detailed instructions for making tissue microarrays and tool set for adjusting the arrayer **(Figure5)**.

New Manual Tissue Arrayer

The new introduced manual Microarrayer, together with pre-made recipient blocks, allows researchers without any substantial cost run this technique in their research group, in the convenience of their own lab without hiring specially trained operator and buying a still semi-automatic machine **(Figure6)**.

Advantages of Tissue microarrays

Tissue microarrays have several significant advantages compared to conventional tumor tissue block sectioning and staining.[6]

1- The most fascinating advantage is tissue amplification. A conventional paraffin block is generally finished after 60 - 80 cuts (depending on the skill of the histotechnologist), resulting in a maximum of 80 experiments per

Figure 3: Tissue microarray Minicore

Figure 4: Automated arrayer (BeecherInstrument)

Figure 5: Manual Tissue Arrayer I (Beecher Instrument)

tissue block. Taking core biopsies allows for a high number of samples to be taken from a tumor block while still maintaining the histologic integrity of the tumor tissue. This significantly increases the amount of experimental work that can be done with each tumor, as each tissue microarray block can be sectioned productively up to 150 times. To date, many papers have been reported using tissue microarrays for evaluation of protein,[7-11] DNA, or RNA expression in high numbers of tissue specimens .[12-13]

2- The second most important advantages are reproducibility and experimental uniformity. Processing tumors in an array results in equal and simultaneous conditions for antigen retrieval and staining reagents. This reduces the natural slide-to-slide variability(in terms of section thickness, identical reagents concentration, temperatures and incubation time) that usually occurs with conventional tissue section processing.

3- Decreased assay volume. Since only one slide contains all of the tumor samples, only small amount of reagent are required for analysis of large number of samples.

4- Other advantages include greater access to automated analysis and computerized data.

5- Maintenance of Tissue microarrays. Generally, tissue microarray blocks can be sectioned approximately 100 to 150 times before the blocks finish. The last sections, although not useful for general analysis, but can be used for tittering or other testing, because 30% to 50% of the cases often still have evaluable tissue.

6- Does not destroy the original blocks. Despite many removed cores from a tumor block, the origin tumor tissue still maintaining the histologic integrity of the tissues showed in **Figure 7** .[6,14]

Tissue microarray validation

There are several criticisms to tissue microarray technology, includingthe issue of representation. This concern includes the problem of tumor heterogeneity regarding protein expression as well as the problem of validity, or the number of essential cores to represent a tumor for assessment of outcome.[14]

Multiple studies have been performed in various tumor types to examine each of these issues.[15-17] Some studies have described the construction, validation, and the use of tissue microarrays applying larger core size of

Figure 6: New Manual tissue arrayer. A. Puncher equipped with tips of 1 to 5 mm in diameter allows you to easily isolate the required core of tissue from a blockand put it into ready to use premade block. B. premade block

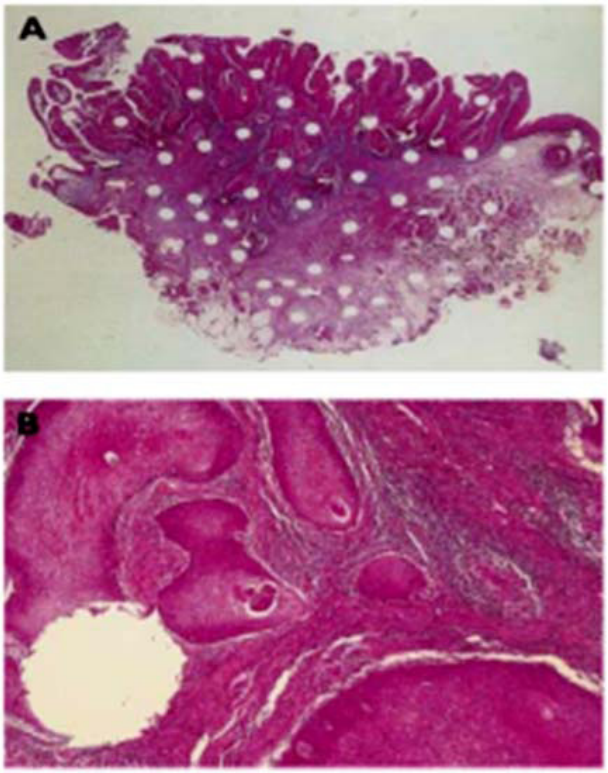

Figure 7: In spite of various removed cores from a tumor block, the origin tumor block still keeping the histologic integrity of the tissue.[6,14]

1 to 2 mm in diameter; however, there is no published evidence that these larger cores are more representative. All of these studies suggest that tissue microarrays, when constructed with exactness along with suitable controls,

can be an accurate method for a high-throughput analysis of markers with minor damage to original tissues.[6]

Controls

The insertion of control tissue in each array is considered essential, but the type of control and the number are very variable. Paraffin-embedded specimens of normal tissue can be inserted as controls in tumor array experiments or analyzed on their own to determine the tissue distribution of normal tissue expression levels.[18] Cell lines,[19] blood,[13] xenografts,[20] synthetic messenger RNA (mRNA) control blocks,[21] and fluid that are prepared as cell blocks can also be embedded in paraffin to serve as controls. Moreover, the whole arrays of control tissues can be used to evaluate antibody activity so that future antibodies can be rated based on comparison of their expression with control arrays.[2]

Tissue microarray visualization

Traditional histochemical, immunohistochemical, DNA, and RNA methods can be used on tissue microarrays by chromogen-based methods, radioisotope-based methods, and flurecence-based methods. Many techniques have been applied for in situ use on tissue microarrays just as they have been used on conventional tissue sections. In addition specific coupling of DNA, mRNA, and protein extraction (for methods such as Western blotting, etc.) from tissue alongside with the construction of tissue microarrays from the same samples can allow direct sample comparison by different detection methods.[22]

Tissue microarray analysis
Traditional, pathologist-based analysis

Traditional semi-quantitative scoring of immunohistochemical staining on whole tissue sections implicates multiplying the percentage of positive cells by the intensity of the staining (% of positive cells × intensity of staining) which usually ranging from 0 to 300.[23-25]

This traditional method for scoring pathology sections, or similar methods based on three- or four- point scale usually cause massive intra- and intro- server variability. However, these methods still used for routine pathology and thus have been applied largely for tissue microarray analysis. As the sections of tissue on TMAs are smaller than whole sections and are often homogenous, less variable area will be in scoring of tissue microaaray samples.[6]

Tissue microarray applications

1.Tissue microarray as validation tools for prognostic/ predictive markers

TMAs have been used by multiple groups to validate a range of targets generated by cDNA microarray or similar technologies. Rubin et al have illustrated this concept in a number of cases in which a prostate-specific marker that was discovered by cDNA array-based analysis was validated using a large cohort prostate tissue microarray.[26]

These large, highly clinically annotated tissue microarrays have also been used for validation of various conventional biomarkers. The main advantage of using tissue microarrays in this framework is the number of assays obtainable from each tissue sample. Because considerable effort is required to collect complete demographic, pathologic, and outcome information, is required to minimized tissue used for each assay. Use of TMAs instead of conventional slides result in 100 to1000-fold amplification of the number of assays per tissue sample.

2. Interobserver reproducibility, tissue processing, and staining variation analysis

Several factors, including different antigen retrieval methods, reagents, and detection methods, can result in different staining in various laboratories. TMAs have been used to rapidly solve the problems of variation in inter-laboratory staining and inter-observer scoring reproducibility in various tissue types such as estrogen receptor (ER) on breast cancer TMAs (inter-laboratory variation)(27)and Gleason grading of prostate adenocarcinoma TMAs (inter-observer reproducibility).[28]

Mengel et al., usedTMAs to examine the influence of 22 different combinations of tissue processing and fixation on Ki-67 staining and its resulting Ki-67 index.[29] They also sent an undistinguishable processed, unstained TMAs to 172 laboratories for independent staining for Ki-67 to evaluate inter-laboratory variability in staining for Ki-67. The stained slides were returned to the laboratory for scoring by two pathologists and for comparison with the preset Ki-67 labeling index for each core. They

found high inter-laboratory variability, with a mean concordance of 75%, whereas very minimal inter-observer variation was seen.

3. Multi-tissue and progression Tissue microarrays

Multi-tissue microarrays are applied to analyzed the level of expression concurrently on various tumor typesand are an excellent source for determining which tumor types express the biomarker of interest. Some of large studies of different tumor type using multi-tumor microarrays have applied both FISH and immunohistochemistry analysis.[30] These tissue microarrays are beneficial for assessing the range of expression among different tumor types. They can also be used to examine which tumor types would be valuable for further study based on their expressions of novel genes that has not previously been studied on multiple tissue types.

A progression tissue microarray consists of different stages and states of tumors all on one slide. This type of tissue microarray has been used in several prostate cancer studies.[31-32] In such TMAs, combinations of samples from normal prostate, benign prostatic hyperplasia (BPH), primary tumors, recurrences, metastases, hormone refractory samples, different Gleason grades, and different stages have been examined concurrently for the expression patterns of particular marker[7,8] Progression studies using tissue microarrays have also been performed in colon cancer,[33-37] gastric cancer,[9] melanoma, breast cancer,[11,38-44] renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), and other tumor types.[10]

4. Clustering and pathway analysis

One of the advantages of tissue microarrays is the easy and fast analysis of multiple pathway components or multiple markers on large number of tumors. Generation of data on tissue microarrays with various markers allows for collecting the results, similar to analysis obtained from cDNA microarray data, to generate biomarker expression-specific clusters, pathway "fingerprints", or disease classifications. A set of 166 breast tumors that had previously been analyzed by genomic hybridization were evaluated by Korsching et al on tissue microarrays.[45] They analyzed HER2 amplification by CISH and 15 other antibodies (including standard breast cancer clinical biomarkers, cyclins, cytokeratins)using traditional immunohistochemitry to look at the relationship between protein expression and patterns of cytogenetic alterations. The semi-quantitative scores of staining on the tissue microarrays were clustered, resulting in three main clusters of tumors including; an HER2 amplification and overexpression cluster, a cytokeratin 8/18 cluster, and a "basal" cytokeratin 5/6-positive cluster, with the different clusters showing higher frequency of expression for different markers. Alkushi et al. using 21 antibodies found that cytokeratins, along with estrogen receptor (ER), vimentin, and carcinoemberyonic antigen (CEA), are the most important cluster components in immunohistochemistry analysis of cervical and endometrial adenocarcinoma TMAs.[46]

Moreover, Hsu et al used a panel of 22 antibodies in a multi-tumor tissue microarray study for the analysis of inter-laboratory variability for S-100 staining in five different laboratories as well as for hierarchical clustering, which was able to classify different tumors based on their site of origin.[47] They adapted tissue microarray data for clustering analysis[48] using software which designed for clustering of microarray analysis by Eisen et al.[49]

Up to now, clustering analysis has been limited by semi-quantitative, discontinuous, and subjective data from pathologist-based scoring of TMAs. Future studies based on data from automated quantitative analysis of TMAs should allow for more robust data sets for complex multivariable analysis.

Pathway analysis has also been performed to examine several members from the same biologic pathway on a single patient cohort. For instance, analysis of the hepatocyte growth factor (HGF)/Met pathway components in node-negative breast cancer TMAs[50] and the PI3K pathway in glioblastoma TMAs.[51] This method may help to find correlations between individual nodes of a pathway as well as multivariate analyses with multiple components to identify pathway signatures.

Future applications

Tissue microarrays are multipurpose and have many other potential future applications. One of the remarkable potential application of TMA is the analysis of expression of different genes or proteins as the goal of target therapy, in a way similar to that used for cDNA arrays.

The potential targets could be identified genes such as genes of particular pathways, functional groups, or other genes related to carcinoma, as well as various unknown genes (expressed sequence tags) for gene discovery on tissue microarrays.

More recently, tissue from transgenic animals is being analyzed using TMAs to allow for rapid and simultaneous assessment of expression of the gene(s) of interest in various tissue types. Tissue microarrays can be also constructed to study cardiovascular, neurologic, adipose, or inflammatory tissue. Cell line and blood as TMAs provide a beneficial way for simultaneous evaluation of biomarkers of interest as controls; therefore, some companies are already marketing cell line arrays. Additionally, TMA spots can be individually microdissected and sequenced for assessment of mutation status of interesting genes.[12] In spite of various applications of TMAs that have been proposed, their use for routine clinical testing is still questionable. However, tissue microarrays have already been applied in routine clinical tests as calibration standards and controls. In summary, it is expected that tissue microarray technology will change the principle standards for protein expression studies, particularly on formalin-fixed, paraffin-embedded tissue.

Tissue microarray in Iran

To get the advantages of tissue microarrays in pathology research, we have established this method in Oncopathology Research Centre, Iran University of Medical Sciences and collected a range of paraffin-embedded tissues from university hospitals.

Our tissue bank includes tissue microarray of breast cancer,[52] prostate cancer,[7,8] lung cancer,[53] gastric cancer,[9] bladder carcinomas,[54] meningioma,[55] pediatric solid tumors[10] and melanomas.[56] These progression TMAsconsist of different stages and states of tumors as well as normal tissue and a control core as hallmark which all have been included on one slide. This series of TMA has been either used or currently being in use in several projects for assessment of various biomarkers including cancer stem cell markers which is a hot topic in cancer research.These data have been either published or are under consideration in peer reviewed journals.We are recently applying TMAs to examine the pattern of protein expression of cancer stem cells compared to pattern of gene expression obtain from cDNA microarray data.

We aimed to collect a broad range of tumors from patients with outcome data to perform high through analysis of biomarkers to find novel molecules for targeted therapy of cancer.

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