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Tissue Microarray: A powerful and rapidly evolving tool for high-throughput analysis of clinical specimens

Anubha Singh, Apurba Kumar Sau

ABSTRACT

This review discusses the various aspects of the Tissue microarray (TMA) technology and its applications in modern molecular and clinical research. Tissue microarrays (TMAs) allow a rapid, cost-effective, high through-put analysis of thousands of molecular markers at nucleic acids or protein level. This high through-put technology enables identification of diagnostic and prognostic markers, in an array of clinical tissue specimens and further translates molecular research to clinical research. A very notable advantage of tissue microarray technology is that it quantitatively amplifies the tissues available for testing, which are normally a limited source in the pathology labs. Various types of tumors, at various stages of disease progression can also be studied at a single platform, in identical conditions in less labor-intensive and cost-effective ways. The current perspectives of this technology in biomedical sciences are promising and researchers can benefit from the enormous data acquired through the use of TMA.

Key Words: Tissue microarray, Tumors, Immunohistochemistry, Quality control

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INTRODUCTION

In the past decade understanding the pathogenesis, progression and treatment of diseases has been revolutionized by using a combination of molecular biology techniques and standard histopathologic procedures [1]. Many novel disease-specific genetic markers and cellular proteins have been identified in the past [2]. Characterization of these markers becomes challenging when multiple markers need to be studied on many clinical specimens using conventional histopathological techniques. Analyses of fresh frozen or paraffin wax embedded tissues for gene and gene clusters or protein markers using routine techniques is cumbersome, time consuming, requires large amount of costly reagents, is affected by laboratory conditions and person-to-person handling and is scientifically less informative and reliable [1]. These limitations can been overcome using tissue microarray (TMA) technology [3]. The purpose of this article is to review the currently known aspects of tissue microarray technology and its application in biomedical sciences.

TECHNIQUES OF CONSTRUCTING TMAs

Battifora et. al. in 1986, described the "multitumor (sausage) tissue block" which they further modified in

1990 as "the checkerboard tissue block" [4]. In the 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. These blocks could be cut to yield multiple sections for various tests. This technique enabled simultaneous examination of multiple tissue specimens in a single experiment, under identical conditions [4]. This technique of constructing TMAs although useful had the disadvantages that only limited number of tissue samples could be incorporated in the block and identification of a distinct tissue sample from the huge "sausage" was difficult.

Almost a decade later, Kononen et. al. in 1998, inspired by the cDNA microarray technology, developed the currently used technique of constructing TMAs [3]. The technique developed by them involved a highly precise holing and sampling process which enables accurate and reproducible localization and further re-localization of distinct tissue specimens. It facilitated precise identification and analysis of multiple molecular markers simultaneously on numerous specimens, in a more organized and consolidated way [3].

Tissue microarrays constructed using the latest instruments, comprise of paraffin blocks in which up to 1000 separate tissue cores can be assembled in an array fashion [5]. The TMAs are built by obtaining cylindrical cores from blocks of paraffin-embedded tissue specimens and arraying them into a recipient block. Before taking tissue cores from the donor blocks, whole sections from donor tissue blocks are cut and mounted on standard microscopic slides. These sections are stained with hematoxylin and eosin [5]. The slides are examined and regions of interest are marked. A hollow needle is used to remove cores from the area of interest from the tissue in the block to construct TMAs. The cores range from 0.6 mm-2.0 mm in diameter. The tissue cores are then placed in precisely spaced manner, at defined array coordinates in the paraffin recipient block. In this way hundreds to thousands of tissue cores can be loaded onto the empty paraffin block. When the tissue cores are being placed at specifically assigned X-Y coordinates in the recipient block, the location and details of the specimen is recorded using a spreadsheet or special softwares. Multiple sections are cut from the recipient block by using a device called microtome. Sections with tissue cores are then mounted on a glass slide. Every single section can be used for independent tests which commonly include immunohistochemistry, fluorescent in situ hybridization (FISH) and mRNA in situ hybridization (mRNA ISH) [4, 6, 7]. The numbers of cores to be arrayed in one paraffin block is selected according to the requirements of the tests. From a single TMA block, containing 1000 cylindrical core specimens, approximately 200 serial sections can be cut, leading to 200,000 test samples.

Investigators can define the experimental array setup according to the 'purpose of study'. Using a multitumor array, diverse tumor types can be sampled from various donor blocks and arrayed on a single recipient block. This array setup provides information on presence or absence of novel molecular markers on diverse tumor specimens. For example, in this type of tissue microarray experimental setup, various tumors such as, lung cancers, breast cancers, gliomas, prostate cancers can be arrayed on a single recipient block [8, 9]. Another array setup includes analyses of tumor progression of one particular type of tumor [10, 11]. In this type of array, cores from one type of tumor but at different grades and stages are put in one block. This gives information on the molecular and morphological changes taking place at different grades and stages of that particular tumor.

USES OF TMAs

TMA technology is a great improvement over conventional procedures of doing tests like immunohistochemistry, FISH and mRNA ISH on each tissue sample separately.

The TMAs have been widely used in cancer research but now this technique is finding increasing application in routine pathology practice and other clinical applications. Using this technique in cancer research, in a single experiment, different types of tumors at various stages of progression, from many different patients can be studied and analyzed simultaneously. TMAs have been widely used for identifying the diagnostic and prognostic markers, therapeutic targets in human tumors and characterization of prevalence of differentially expressed genes as reviously identified by cDNA microarray technology [12, 13, 14, 15]. TMAs are also being used as a popular tool to study the expression patterns of putative tumor suppressor genes and for identifying genes that are targets of chromosomal amplification [16, 17].

Immunohistochemistry (IHC) is the most routinely practiced regimen for analyses of various histological samples including tumor identification. One of the major problems routinely encountered in IHC is 'quality control' [18, 19, 20, 21]. Quality control for research and non-research purposes is urgently needed because an increasing number of therapeutic regimens are based on IHC staining results for specific proteins (for example, the estrogene receptors, erbB2, epidermal growth factor receptor and c-kit) [1]. A high variability in staining results is observed in intra-laboratory and inter-laboratory tests [1]. This may happen due to differences in antigenic epitopes, batch to batch variability in antibodies, differences in staining procedures and in observation and interpretation of staining results [1]. Tissue microarrays overcome these factors and promises highly reliable quality assurance for immunohistochemistry.

TMAs can also facilitate the standardization of immunohistochemical staining procedures. Standardization of antibodies and reagents used for immunohistochemistry is a time consuming work. Different tissues which are positive and negative for an antibody are tested at multiple dilutions and retrieval times during standardization. With the use of serial

sections of a control TMA block with multiple tissues with a range of antigen densities, variations in staining results can be discovered immediately, regardless of whether they are caused by differences in reagents or variations in the staining procedure. It is possible to devise TMAs with cores arranged in 5x5 pattern (25 different tissues) taking only 4x4mm space enabling the determination of a very wide antigen spectrum. Standardization using these TMAs could also be applied to other tests like fluorescent staining methods and brightfield in situ hybridization [22].

ADVANTAGES OF TMAs

- 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 and in a cost effective way [3].

- Large number of samples can be rapidly analyzed at the same time and statistical significance of new markers can be precisely determined in a single experiment [23].

- Any error in a given test sample can be taken care of by the statistical analysis of hundreds or thousands of samples, which eliminates any effect of variability due to individual data point and makes it easier to conclude the results [23].

- Intact tissues, cell culture materials and cell lines, all may be used for constructing TMAs. This provides a semiquantitative evaluation of the expression of proteins or genes of interest because the absolute expression levels of these can be determined.

- Mini TMAs with 16-25 cores can be used as internal controls when performing immunohistochemistry, FISH and other tests to provide a high degree of intra-laboratory reproducibility. The test tissues and the tissue of interest are stained under the identical conditions. The small size of the tissue core diameter reduces the consumption of expensive antibodies and other reagents compared to external controls on separate slides [5].

- The information obtained by using TMAs is more scientifically informative and holds greater potential for reducing the time and cost of research in tissue banking, proteomics, and outcome studies.

- TMAs greatly amplify the quantitative analysis of tissues, which are normally a limited source in the pathology labs. The number of sections that can be obtained from a standard archived block are 50-100, whereas, from this same tissue block, 200-300 core biopsies could be taken and subsequently 1000's of sections can be cut from this microarray block, generating material for 10, 000's of assays.

- Using TMAs ensures identical treatment for all the samples. Since all the samples are treated in a similar manner and under similar experimental conditions, it is less subjected to experimental and technical disparities.

- TMAs can be used for techniques such as immunologic stains with either chromogenic or fluorescent visualization, fluorescence or mRNA in situ hybridization, tissue micro-dissection techniques and 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.

- TMAs preserve the original tissue samples, which could be used later for other diagnostic purposes thus conserving the valuable original tissue sample.

- TMAs confer less inter and intra-laboratory differences, since it facilitates standardization of key molecular assays such as immunohistochemical, fluorescence and mRNA in situ hybridization, giving more reproducible results.

- TMAs can provide possibilities for high through-put molecular profiling of clinical human tumors such as, lung cancers, breast cancers, gliomas and prostate cancers. The technique can be used for validation of new molecular markers on histopathologic tissue specimens.

- As researchers are now able to analyze hundreds of tissue samples on a single glass slide using the tissue microarray technology, the information stored in a single glass slide is enormous involving approximately Gigabytes of data. Many investigators studying the same diseases at different institutions might benefit from the sharing of these results.

LIMITATIONS IN USING TMAs

Sample fixation and embedding has great impact on the quality of TMA sections. Most commonly used tissue fixative in histopathology laboratory, buffered formalin, modifies the RNA molecule by adding mono-methyl groups to its base which has a likely damaging effect on

RNA by altering antigenic epitopes structure [24, 25]. This may give erroneous results of *in situ* analysis of DNA, RNA and proteins. To circumvent this problem, tissues can be fresh frozen at -70 deg C, without using any chemical fixatives [26, 27]. This approach preserves the integrity of DNA, RNA and proteins, however, fresh frozen tissue loose its architecture and cellular morphology as compared to the formalin fixed paraffin-embedded tissue array [28].

Small size of the TMAs may not provide a glimpse of the entire tissue profile. In certain heterogeneous cancers, such as prostate adenocarcinoma and Hodgkin lymphoma, small cores may not be representative of the whole tumor [5]. An original tumor tissue could be upto several centimeters in diameter whereas the tissue cores range from 0.6mm to 2.0mm in diameter. A tumor tissue may comprise of many different histological areas within itself, such as regions of apoptosis, necrosis or increased proliferation etc and it may not be possible to sample all areas in one tissue core [1]. Care should be taken while taking the cores from the original tissue and focus should be made on the 'purpose of investigation'. As is well documented, the grading of breast cancer is dependent on presence and number of mitosis. As the proliferation of tumors is maximum at the periphery therefore breast cancer arrays studying tumor proliferation markers should be punched from the periphery of the original tumors [5]. Also, while studying very complex tissues, multiple core samples should be taken from various areas of the tumor block covering entire spectrum of the pathology. It may also be possible to use large bore needles for taking tissue cores. Several studies have validated that results obtained from analysis of tissue microarray cores and whole sections using immunohistochemical studies are identical and comparable. Parker et al, showed good concordance between results obtained from whole tissue sections with that of tissue microarray in quality assessment of estrogen receptor status in breast cancer in majority of the cases [29]. Many other such quality assurance studies have demonstrated that the intratumoral heterogeneity do not limit the effectiveness of TMA as a tool for molecular pathology studies.

PROBLEMS IN ROUTINE USE OF TMAs

The present TMA technology still has scope for its upgradation and more advanced versions of the current TMA technology will be required in future. Control over holing and sampling 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 [30].

Automation and digital techniques have immensely revolutionized clinical medicine and biomedical research, and now the digital techniques are becoming part of every pathology laboratory. TMA technology is a powerful technique for use in biomedical research, especially cancer research. To make this technique more powerful in terms of ease of performance and data acquisition, there is a need to digitalize the TMA technology [6]. Digital image analysis technology that can scan tissue microarrays into "virtual slides" with high resolution and that can analyze images algorithmically will accelerate the discovery of new predictive biomarkers.

One of the major drawbacks of TMA technology is the high cost of TMA facilities. The high cost of the array machines limits its use in general practice in many countries. The tissue microarray machines from Beecher instruments, San Prairie, Wisconsin, USA costs anywhere from 12,000 USD to 42,000 USD [31]. Even the manual ones cost around 1200 USD, which are not be affordable to many laboratories in developing countries. Efforts have been made by many researchers to devise more cost effective TMA construction techniques [31-36].

FUTURE PERSPECTIVE

The era of tissue microarrays has just begun. A multitude of different possibilities exist of which some are already in use. For example, the use of "paraffin wax tissue banks" in pathology departments for the retrospective evaluation of new tumor markers for individual patients is a novel modality. Prognostic factor dependent treatment modalities which are being discovered and implemented in daily clinical practice and "predictive molecular pathology" require new methods to enable a retrospective patient tailored characterization. It will only be a question of time before TMAs find their role in educational purposes. Nevertheless, the major focus of TMAs at the present time is in the fields of cancer and non-cancer research. The widespread use of TMAs will become an integral part of daily practice in research and routine clinical laboratories. With this clear perspective, 'pathology' as an old, largely morphology based medical specialty will find itself in a central position within these new developments.

In order to get benefited from this TMA technology and to get an open access to this enormous data, scientific community needed an open source TMA data exchange specification that will convey all of the data in a TMA experiment in a format that is precise and understandable. In May 2001, Association of Pathology Informatics (API) [37] developed an open, community-supported TMA data exchange specification for the first time [38]. This allows researchers to submit their data to journals and to public data repositories and to share or merge data from different laboratories.

CONCLUSION

The invention of TMAs and commercialization of this technique is a boon for scientists and pathologists who, without this technique, would spend too much time studying numerous tests done by conventional methods. TMA has numerous potential applications in basic research, drug discovery and prognostic oncology. It has potential to become a widely used tool in tissue related

research and its fast applicability will accelerate and push forward the transition of basic research findings into clinical applications.

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Anubha Singh - Conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Critical revision of the article, Final approval of the version to be published
Apurba Kumar Sau - Conception and design, Drafting the article, Critical revision of the article, Final approval of the version to be published

Guarantor
The corresponding author is the guarantor of submission.

Conflict of Interest
Authors declare no conflict of interest.

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