DOI: 10.7860/NJLM/2018/31639:2271

Review

Pathology Section

Tissue Microarray Technology- A Brief Review

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ABSTRACT

In this era of modern revolutionisation in the field of medical laboratory technology, everyone is aiming at taking the innovations from laboratory to bed side. One such technique that is most relevant to the pathologic community is Tissue Microarray (TMA) technology. This is becoming quite popular amongst all the members of this family, right from laboratory scientists to clinicians and residents to technologists. The reason for this technique to gain popularity is attributed to its cost effectiveness and time saving protocols. Though, every technique is accompanied by disadvantages, the benefits out number them. This technique is very versatile as many downstream molecular assays such as immunohistochemistry, cytogenetic studies, Fluorescent In situ-Hybridisation (FISH) etc., can be carried out on a single slide with multiple numbers of samples. It is a very practical approach that aids effectively to identify novel biomarkers in cancer diagnostics and therapeutics. It helps in assessing the molecular markers on a large scale very quickly. Also, the quality assurance protocols in pathological laboratory has exploited TMA to a great extent. However, the application of TMA technology is beyond oncology. This review shall focus on the different aspects of this technology such as construction of TMA, instrumentation, types, advantages and disadvantages and utilisation of the technique in various disease conditions.

Keywords: Molecular pathology, Tissue bank, Tumour, Tumour diagnostics

INTRODUCTION

Several innovative developments have been generated for the diagnosis and treatment of cancer over the past decade. Many number of new approaches for analysing alterations at both gene and protein level are being introduced for research and routine laboratory practise. Novel tools are available to ease the interpretation of huge sets of data within a short period of time. Use of TMA technology is one such tool which is revolutionising in clinico-pathological research setting. The basic concept of this technique is to scale down the available intact tissue for high-throughput molecular analysis. The potential applications of this tool are highly increasing beyond horizons and are becoming popular amongst researches, pathologists and basic scientists.

A novel method called the Multi-tumour tissue block-sausage technique was described in 1986 [1]. However, the drawbacks of this technique were addressed by other researchers [2] and came up with the so called TMA technology used in modern research. They introduced a highly precise instrument for punching by which the samples could be exactly placed and re-localised. TMA has been a promising solution for creating a tissue bank comprising of paraffin wax blocks for evaluating and discovering novel biomarkers retrospectively of individual patients which was once thought to be an impossible task. This technique is also known as tissue chip [3].

Construction of TMA

The success of a TMA analysis depends upon many factors such as selection of formalin fixed paraffin embedded tissue blocks/frozen tissue blocks, respective Hematoxylin and Eosin slides, marking the area of interest, punching of the appropriate area on the donor blocks, relocalizing the punched bit on the recipient block. Once the donor blocks are selected and reviewed, then one starts with the array construction. This may be manual or automatic. The data and the order of the tissues on the recipient block is usually documented in an excel sheet. For convenience, one row at the beginning or at the end consists of one extra core of the tissue for referencing purposes [4].

In most of the literature that is available the authors have used the instruments manufactured by Beecher's instruments, San-Prairie, Wisconsin, USA [4]. There are various tissue core needles available with diameters ranging from 0.6 to 2.0 mm. While punching a pre-defined region from the donor block, corresponding Hematoxylin and Eosin slide is placed below for orientation. Cores of tissue sections are then transferred onto a recipient block which is then sectioned onto a glass slide for further analysis.

The size of the core is still debatable. While many researchers feel 0.6 mm diameter is good enough for conducting analysis and a single recipient block could accommodate several

hundreds of tissue. Logically, 2 mm diameter core tissue shall accommodate less number of cores but will be able to provide much larger area for analysis from the parent block. It has been postulated from studies that though the core diameter of the TMA samples may vary from 0.6-2.0 mm, this core diameter considered more so looks to be insignificant as compared to the original size of the tumour which ranges up to few centimetres. Hence, it is the site from where the sample has been punched is what determines the strength of the diagnosis than the density of the core sample taken. For example, in breast cancer the number of mitotic cells is more in the periphery than in the centre of the tumour [5]. Therefore, the sample punched at the periphery is more diagnostic irrespective of the size of the sample taken for TMA analysis. Further, lesser the diameter of core tissue, more number of tissue cores can be analysed on a single recipient block. However, larger diameter of core tissue may be required when conducting research on complex tissues (small benign/ malignant) where various regions in an organ have to be investigated simultaneously. When 2 mm punchers are used one can achieve arrays upto 50-100 specimens while 0.6 mm punchers can yield 300-500 specimens allowing a spacing of 0.8 mm per block using regular tissue cassettes [6]. However, even 1000 samples cores can be accommodated in a single recipient block with a core diameter of 0.43 mm [7]. This allows conservation of tissue in the donor block without damaging the tumour structure and may be used for conventional sectioning if the need be. Few investigators have reported 10-30% loss of tissue during construction of TMA (manual/automated) [8-10].

Shi Y et al., used an alternative method and constructed the array by obtaining 'tissue rods' from donor blocks (gastrointestinal stromal tumours) and created a recipient block consisting of 88 small lattices [11]. The recipient blocks containing rods were embedded vertically. This method being different from the conventional block construction where usually cylindrically punched cores are used. This promises to obtain improved and high output sections from the resulting TMA block.

Shebl AM et al., demonstrated the use of another method to obtain cylindrical tissue cores using mechanical pencil technique, alternative to the use of punchers [12]. This has been the most inexpensive and easy method available in literature and may be used by most of the developing countries because of its cost effectiveness.

Incorporating automation in the technique will increase the punch speed and block capacity by 7 folds. One will be able faster in the construction and can mark, edit and save the co-ordinates of the punches with the help of an on screen display and software. Visual selection is also possible using a magnifying glass or a stereomicroscope [13].

Types of Arrays

The types of arrays are highly variable [14,15].

1. Normal tissue arrays

2. Non-neoplastic/non-cancerous arrays (eg., diabetes, renal transplantation [16], autism [17] , neurodegenerative diseases [18], dermatology [19], placental diseases [20], cardiac [21]).

3. Neoplastic to cell lines (cell line microarrays) [22].

The neoplastic arrays are also referred to as tumor TMAs and are further classified as [23-25]:

I Multi-tumour arrays

II. Progression arrays (based on tumour stage)

III. Prognostic arrays (known clinical end points)

I. Multi-Tumour arrays: This type of arrays can be used to screen large number of tissue from varied tumour types and it is possible to array them on a single recipient block. It shall be advantageous in analysing presence or absence of novel biomarkers pertaining to the tissue samples [23].

II. Progression arrays: This type of arrays are used to assess the histological and molecular changes at various stages of a particular tumour type. It may be carried out right from a localized disease to metastasis. It may be useful in analysing the expression of certain proteins at different stages of the tumour development which may also supplement to know the aggressiveness of the condition.

III. Prognostic arrays: The association of patient outcomes to data obtained using TMA analysis has been an area of interest to the clinicians. This kind of associations has been studied in prostrate carcinomas.

Advantages

This innovative technique has greatly proved its usefulness in the field of pathology. Retrospective analyses of huge data sets on Formalin Fixed Paraffin Embedded tissues (FFPEs) are now possible within a short duration and are greatly cost effective. Also it's possible to carry out the analysis using very less archival tissue. Large numbers of tissue cores are being subjected to uniform conditions and are helpful for validation and discovery of potential biomarkers. Rapid generation of results by FISH using TMA to study oncogenes such as CCD1, CMYC, ERBB2 in 397 cores has been proved. The simultaneous analysis of large sets of data considerably improves the precision and power of statistical analysis. The identical conditions for all the cores of tissue samples analysed ensure that there is no batch to batch variability as with conventional sections [26,27].

In Immuno-Histochemistry (IHC), TMA's can be used for inter laboratory quality control. The reduction in the use of antibodies and reagents proves that this technique is economic in terms of cost. The novel prognostic biomarkers arising from cDNA microarray analysis can be validated using TMAs [28]. Natkunam Y et al., analysed 1335 tumours to test a novel marker called MUM1/RF4 of plasmacytic differentiation and concluded it to be sensitive over a non-specific marker [29]. The techniques such as FISH, complimentary DNA (cDNA), comparative genomic hybridization may be used in combination with TMA or TMA slides itself may be used to

carry out these downstream molecular analysis to potentially explore the molecular basis of cancer development and progression [2,28,30,31].

In addition to histomorphology, biomarker expression analysis (IHC), TMA can be used to assess amplification studies of DNA, RNA and proteins. This technique facilitates in identification of molecular targets, prognostic and diagnostic markers in tumours [26,32-34].

In routine surgical pathology incorporating a single tumour of all grades in a single slide will enable to master the art of tumour grading. Thereby, the variability of grading the tumours between the observers will be reduced [35].

The field of bio-marker research can further be escalated by integration of TMA technology with digital pathology [36].

Disadvantages

The most important disadvantage of this technique is that one small tissue core may not be representative of whole tumour analysed conventionally. Therefore, many such cores of the same may be required to carry out analysis to arrive at a definitive conclusion. This is mainly significant for heterogeneous tumours like human ovarian tumour [37] etc. Whether this technology is useful in heterogeneous tumours is still highly debated.

Rosen DG et al., have shown that there are 91% chances of one core being representative of the whole section; 97% with two cores and 98% with three cores [37]. But, Zhang D et al., successfully concluded that there was no significant difference between results obtained by conventional sectioning with that of TMA with regards to pathological parameters and that a single core for each specimen ensured representative tumour without sampling bias in breast tumours [38].

Future Perspectives

This technique can be supplemented with automation which shall further reduce the burden on the personnel and generate more precise information. Archival of many scarce tissues on a single block and digitisation of the information can help in conservation of huge data sets for decades which may be useful to carry retrospective analysis of novel markers. This in turn will immensely help in the expulsion of endless searches for archival material needed to conduct the experiments.

CONCLUSION

Few scientists consider the evolution of TMA technology as the most note worthy innovation amongst the others in the field of histopathology over the last decade. This powerful platform plays an important role in bridging the gap between the laboratory and the bed side. It shall also benefit the students and researchers greatly. It shall open horizons to study various gene and protein expression patterns in wide range of normal, cancerous and non-cancerous tissues.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Publishing: **Jan 01, 2018**