Tissue microarray- A review

Nagaraja A[1], Rashmeet Kaur[2,*], Richa Bansal[3], Sujata Saxena[4], Bhavana Rai[5]

[1]Professor and Head, [2]PG Student, [3,4]Reader, [5]Senior Lecturer, Dept. of Oral Pathology, Seema Dental College and Hospital, Rishikesh, Uttarakhand, India

***Corresponding Author: Rashmeet Kaur**
Email: drrashmeetdharni@gmail.com

Abstract
Cancer is a highly variable disease with multiple heterogeneous genetic and epigenetic changes. Functional studies are essential to understand the complexity and polymorphisms of cancer. Tissue microarray (TMAs) is a powerful new technology designed to assess the expansion of proteins or genes across large sets of tissue specimens assembled on a single glass microscope slide, efficiently and economically. In this review, we are highlighting the different aspects of technology such as contribution of TMA, advantages, disadvantages and utilization of technique in the diagnosis of oral cancer.

Keyword: Tissue microarray, Oral cancer, Head and neck squamous cell carcinoma.

Introduction
Recent advances in the field of human molecular genetics have revealed gene based disease mechanisms in many area 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.[1]

The idea of studying a large number of formalin fixed and paraffin wax embedded tissues simultaneously in a single histologic section is not new. Various techniques have been proposed such as In-situ hybridization, Fluorescent in situ hybridization (FISH), immunofluorescent technique, Sausage technique etc. The major drawbacks of these previously described techniques were the limited number of tissue samples that could be included and the problems in identifying a distinct tumor or tissue sample in the big sausage. These limitations have been overcome by using tissue microarray technology (TMA).[2]

J. Kononen et al (1999) developed the TMA technique.TMAs are the paraffin blocks composed of multiple specimens, which can be simultaneously investigated with different in situ techniques under identical laboratory conditions, resulting in dramatic time and cost reduction compared with conventional pathologic studies.[3]

Principles of TMA
1. Prevalence TMA – are assembled from tumor samples of one or several type without attached clinical and pathological information. These TMAs are used to determine the prevalence of a given alteration in the areas of interest in a tumor.
2. Progression TMAs – contain samples of different stages of one tumor type. They are used to discover association between tumor genotype and phenotype**.**
3. Prognosis TMAs – contain samples from tumors available with clinical follow-up data. They represent a fast and reliable platform for the evaluation of clinical importance of new detected disease related genes.
4. Experimental TMAs – are constructed from tissue like cell lines. Cell line TMAs are optimally suited for screening purposes, e.g. tumor samples from TMA archives are also used in it.[4]

Construction of TMA
TMAs are method of relocating tissue from standard histological paraffin blocks such that tissue from multiple donor blocks can be placed on the same recipient block. Therefore, the first step in the process of creating a good TMA block is to locate the representative area on each Hematoxylin and Eosin slide and careful identification of the same area on the corresponding paraffin block of the slide. Once this is done, the block is positioned underneath the TMA puncher such that the representative area is directly under the punching pins. Thereafter, small core needle biopsies of these representative tissues are punched out directly from donor paraffin blocks and re-embedded onto a new recipient TMA paraffin block. Many such cores can be embedded in such a master arrayer block employing this technique.[5]

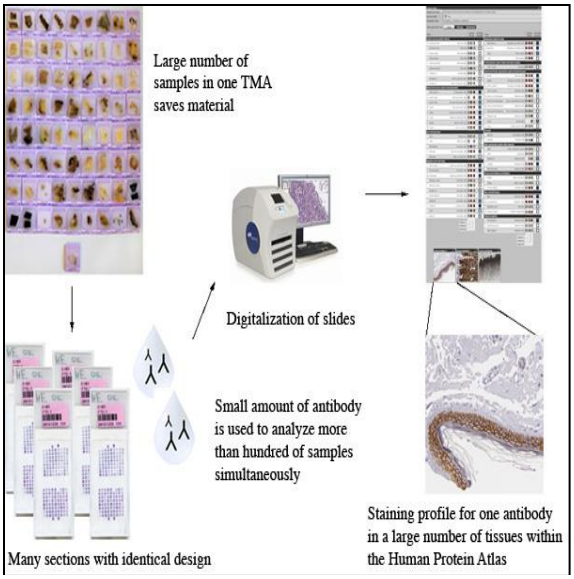

Fig. 1: Overall scheme of the process within the human protein atlas Uppsala

Fig. 2: Donor stylet marked for standardized length of tissue cores eg 4.5 mm

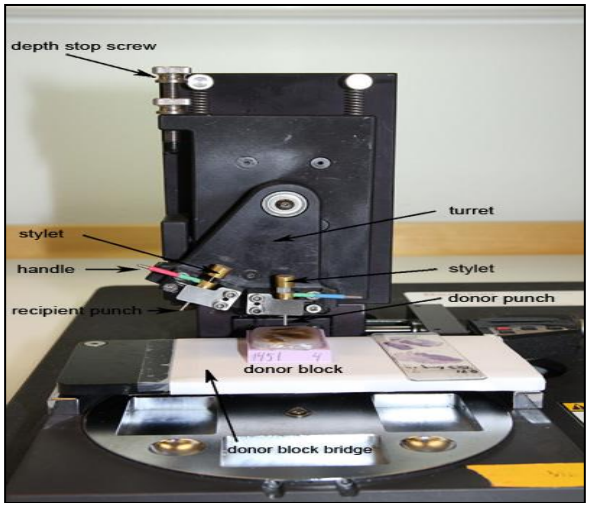

Fig. 3: Mannual tissue microarrayer

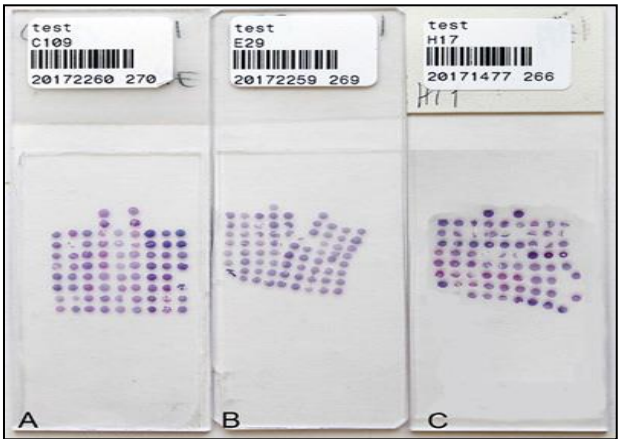

Fig. 4: Hematoxylin and eosin stained TMAs. (A) A properly cut section with straight and aligned columns and rows. Using a bad or dirty blade could result in splitting (B) or compressed sections (C)

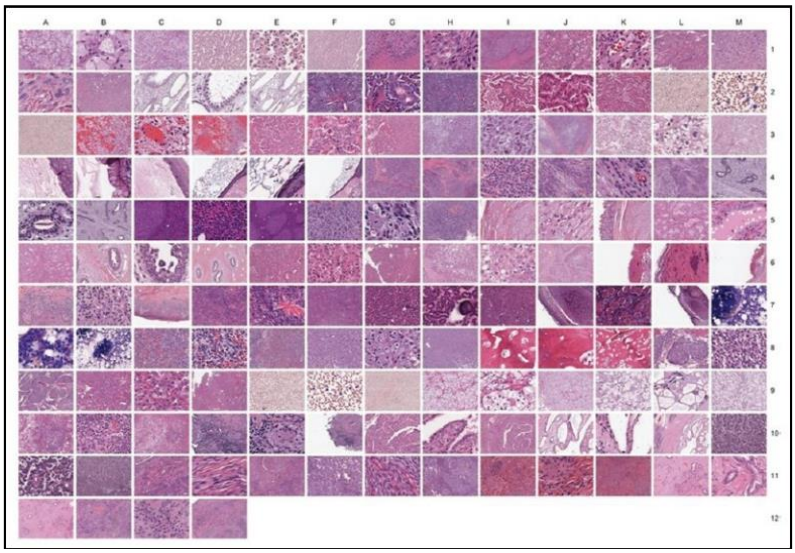

Fig. 5: An IMA was created from the University of Michigan, Department of Pathology WSI library: Various tumors were used to create this digital slide IMA

Technique of TMA

There are two types of TMA technique, automated and manual. First is automated arrayer which is easy to use and includes a specimen tracking software system. The instrument marks, edits, and save punch coordinates using an onscreen display and software tools. It pre-marks the punch area. The video merge until displays pre-marked slide images side by side to the donor block image. Some 120 to 180 cores can be punched per hour. The automated arrayer is deal for a laboratory with a high volume of TMAs. Second is manual arrayer which includes visual selection while punching depends on the technologist, who uses a hand-held magnifying glass or magnifying lamp attached to the counter or a base to hold the magnifying lamp in place. The pathologist marks all areas of interest on the slide and the technologist makes the movements. Cores punched per hour depend on the experience of the worker. The average number of cores punched per hour using the manual arrayer is 30 to 70. To prepare microarray blocks for special stain controls, or QC controls for H and E staining, an inexpensive pen extractor would be suitable.[6]

Uses of TMA

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 previously identified by cDNA microarray technology. 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. TMAs can also facilitate the standardization of immunohistochemical staining procedures. Standardization using these TMAs could also be applied to other tests like fluorescent staining methods and bright field in situ hybridization.[7]

Advantages of TMA

Researchers can study an entire cohort 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. 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. Any error in a given test sample can be taken care of by the statistical analysis of hundred or thousand of samples, which may eliminate any effect of variability due to individual data point and make it easier to conclude the result. Mini TMAs with 16-25 cores can be used as internal controls. The small size of the tissue core diameter reduces the consumption of expensive antibodies and other reagent compared to external controls on separate slides. The information obtained by using TMAs is more scientifically informative and holds greater potential for reducing the time and cost for research in tissue banking, proteomics and outcome studies. TMAs preserve the original tissue samples, which could be used for later for other diagnostic purposes thus conserving the valuable original tissue sample.[7]

Limitations of TMA

Small size of the TMAs may not provide a glimpse of the entire tissue profiles. In certain heterogeneous cancers such as prostate adenocarcinoma and hodgkins lymphoma, small cores may not be representative of the whole tumor. One of the major drawbacks of TMA technology is the high cost of TMA facilities. The high cost of the array machines lits its use in general practice in many countries.[6]

Troubleshooting and Tips

1. Core does not come out of the punch easily – punch tip is bent or distorted. Change the punch.
2. Tissue core was pushed too deep – remove the sample with the small punch and place a new sample in the same position.
3. Insufficient spacing of cores – can cause minor cracks or stress on the core when sectioning.
4. Thinning of TMA core in block – this is the result of repeated sectioning of the same block where cores are uneven in block.
5. Loss of tissue on water bath – due to folds, wrinkles and mishandling of ribbon.
6. Re-facing block – when sections are cut to accommodates slides for stain requested. If the block is filed and then pulled for cutting of extra slides, the block is repositioned and re- faced. This is why it is important to use a dedicated microtome.
7. Re-facing angle – shortens the life of the tissue micro-array block which is called thinning. Make sure the cassette is completely flat on top of the mold[8].

Microarray in Oral Cancer

To date, only a few microarray studies relevant to oral cancer have been published. Chang et al. (1998), illustrated the use of cDNA microarrays to characterize transformation-related genes in oral cancer. Villaret et al. used a combination of complementary DNA subtraction and microarray analysis to evaluate unique genes specific for squamous cell carcinoma of the head and neck (HNSCC) as potential tumor markers and vaccine candidates. Nine known genes were found to be significantly overexpressed in HNSCC as compared to normal tissue. Villaret et al. (2000), in addition four novel genes were overexpressed in a subset of tumors.

Alevizos et al. (2001), analysed the transcriptome in oral cavity squamous cell carcinoma. They found about 600 candidate genes (oncogenes, tumor suppressors, transcription factors, differentiation markers, metastatic proteins and xenobiotic enzymes)

that were differentially expressed in oral cancer, validating only three of these genes by PCR.

In a very recent study, Leethanakul et al. (2003), generated high-complexity cDNA libraries from laser capture microdissected normal and cancerous squamous epithelium. In this study, the authors surveyed the available sequence information using bioinformatic tools and identified 168 novel genes differentially expressed in normal and malignant epithelium. Moreover, using cDNA arrays, they obtained evidence that a subset of these new genes might be highly expressed in HNSCC.[9]

Future Prespective

The era of tissue microarrays has just begun. A multitude of different possibilities exist of which some are already in use. Prognostic factor dependent treatment modalities which are being discovered and implemented in day clinical practice and predictive molecular pathology require new methods to enable a retrospective patient's tailored characterization. It will only be a question of time before TMA finds their role in educational purposes. Nevertheless, the major focus of TMAs at the present time is in the field 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 speciality will find itself in a central position within these new developments.[10]

Conclusion

The invention of TMAs and commercialization of this technique is a boon for scientists and pathologist who, without this technique, would spend too much time studying numerous test done by conventional methods.

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