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Review Paper

Tissue Microarray Technology a Novel research tool for Oral Squamous cell Carcinoma: A Review

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Abstract

Over the last few decades several new high throughput methods have been introduced in research for analysing the expression of a wide range of proteins that have an enormous value in diagnosis and treatment of human cancers. The Tissue microarray technology is one such novel research tools. Tissue microarray technology is a method of takingmultiple tissue cores from conventional histological paraffin blocks and placing them in a single paraffin block. Thus, the tissues from multiple patients can be seen on the same slide. It is a means of combining hundreds of tissue specimens onto a single slide for simultaneous histological analysis. This technique is grown to become a population level research tool. TMA hold an immense potential to validate multiple tumor types in a shorter time span. Tissue microarray represents a mechanism for highly effective use of scarce resources. This paper presents an overview of this novel technology, the benefits and challenges of this technique and traces the application of a combined approach of Immunohistochemistry and tissue microarray in research pertaining to oral squamous cell carcinomas.

Keywords: Tissue microarray, oral squamous cell carcinoma, amplification, molecular markers, prognosis.

Introduction

For centuries pathologists have relied on hematoxylin and eosin stained Formalin fixed paraffin embedded tissue sections for diagnosis of human diseases, and it continues to remain as the gold standard for diagnosis. Over the years as the understanding of the disease process grew so did the development of diagnostic modalities. One of prominent discoveries of this decade is the tissue microarray technology (TMA). This technique is efficient method to preserve tissue samples.

The TMA technology had its beginning in 1986, when Hector Battifora introduced a ‘sausage' block method. In this method 1-mm thick ‘rods' of tissue, obtained from different specimens, were wrapped carefully in a sheet of small intestine. This sausage of tissue was embedded in a normal-sized paraffin block, which was then called the 'multitumor tissue' block. This method was designed for immunohistochemical control and standardization between laboratories[1].

In 1987, Wan et al modified Battifora’s “sausage” block method whereby tissue cores were placed in specific spatially fixed positions in a block. A few years later, in 1990, Battifora improved the method to a 'checkerboard' arrangement of the “tissue rods”. The tissues were evenly distributed in a checkerboard fashion and the tissues could be readily identified by their position in the resulting sections[2]. Although these methods conferred a significant advantage of simultaneously examining multiple tissue specimens under identical conditions, one could not satisfactorily identify individual tissue ‘rods’.

Almost a decade later, in 1998, JuhanKononen and his associates, extended the idea originally proposed by Battifora, and developed the tissue array technology, which is currently in practice. This technique employs a novel sampling approach to produce tissues of regular size and shape to be densely and precisely arrayed on a single paraffin block[3]. Very quickly, this technique became a prominent tool in cancer research.

TMA is essentially a simple mechanical method wherein minute cylindrical tissue cores of regular shapes and size are taken from FFPE “donor” tumor blocks and are subsequently arrayed on a “recipient” - TMA block[4,5] (figure 1).

TMA is a method of relocating tissue samples from multiple standard histological paraffin blocks and inserting them onto a single recipient paraffin block. To achieve this, a hollow needle is used to remove tissue cores from regions of interest in paraffin embedded tissues such as clinical biopsies or tumor samples. These tissue cores are then inserted into a recipient paraffin block in a precisely spaced, array (grid) pattern. Sections from this block are cut using a microtome, mounted on a slide and then analysed by any method of standard histological analysis. Each microarray block can be cut into 100-500 sections which can be subjected to independent tests[3].

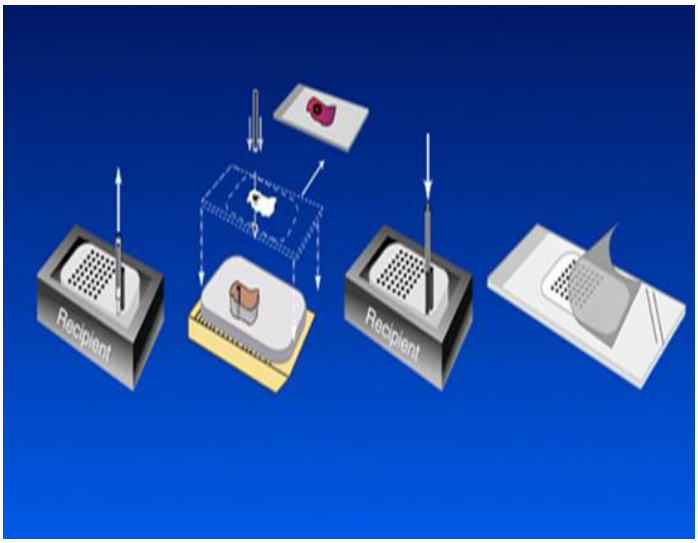

Figure-1
Principle of tissue microarray technology[3]

Construction of Tissue microarrays

Tissue microarrays are constructed from a range of precision instruments that are manual tissue microarrayers, semi-automated or fully automated microarrayers[6,7,8] (figure 2, figure 3, figure 4). The most popular tissue arrayers are those designed by Beecher's instruments (San Prairie, Wisconsin, U.S.A)

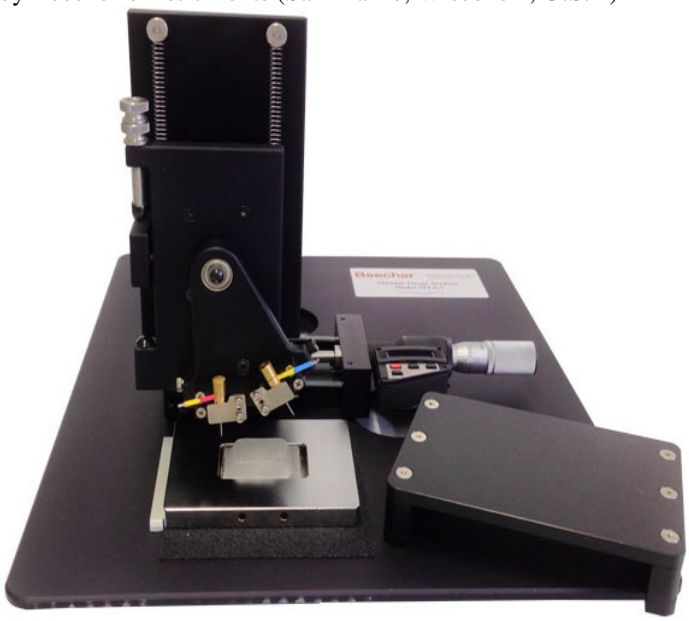

Figure-2
Manual Tissue Microarrayer[6]

Preparatory work

The first step in construction of a tissue array block is to design the study. The Formalin fixed paraffin embedded tissue blocks (Donor blocks) and the respective hematoxylin and eosin (H&E) stained slides of all the selected cases are set apart.

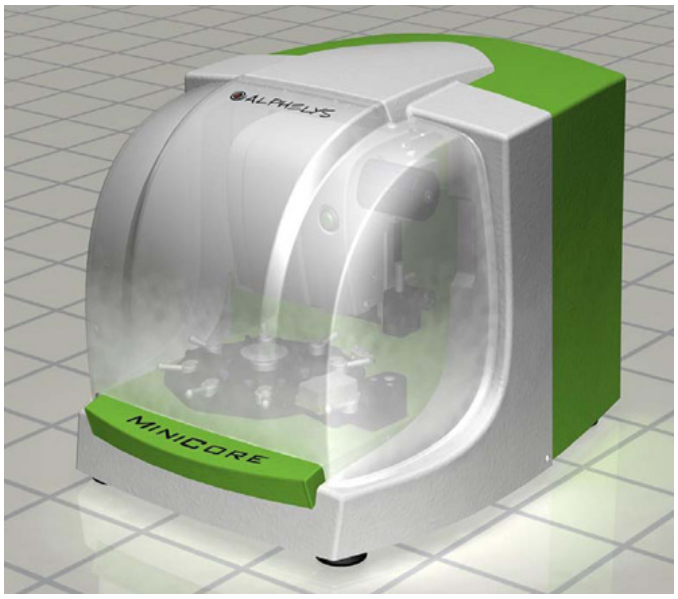

Figure-3
Semi automatic Microarrayer[7]

Figure-4
Automated tissue microarrayer[8]

A new section is cut from the donor blocks and stained with hematoxylin and eosin (H&E). All these stained sections are reviewed and the areas of the tumor where the cells are least differentiated should be marked. For each case a minimum of 2 areas should be identified. The marked micro slides are superimposed on the respective FFPE tumor block and the corresponding area on the tumor sample is labelled (figure 5).

Meanwhile the recipient paraffin block of dimensions 45X22 mm should be prepared. The recipient block is usually prepared with paraffin wax that is softer than the regular wax, usually a wax with a melting point of 56^0C. The surface of the block is gently trimmed to make it smooth and of a uniform plane. Additionally, a custom made Excel spread sheet for recording the core positions is prepared and kept in the working area[5].

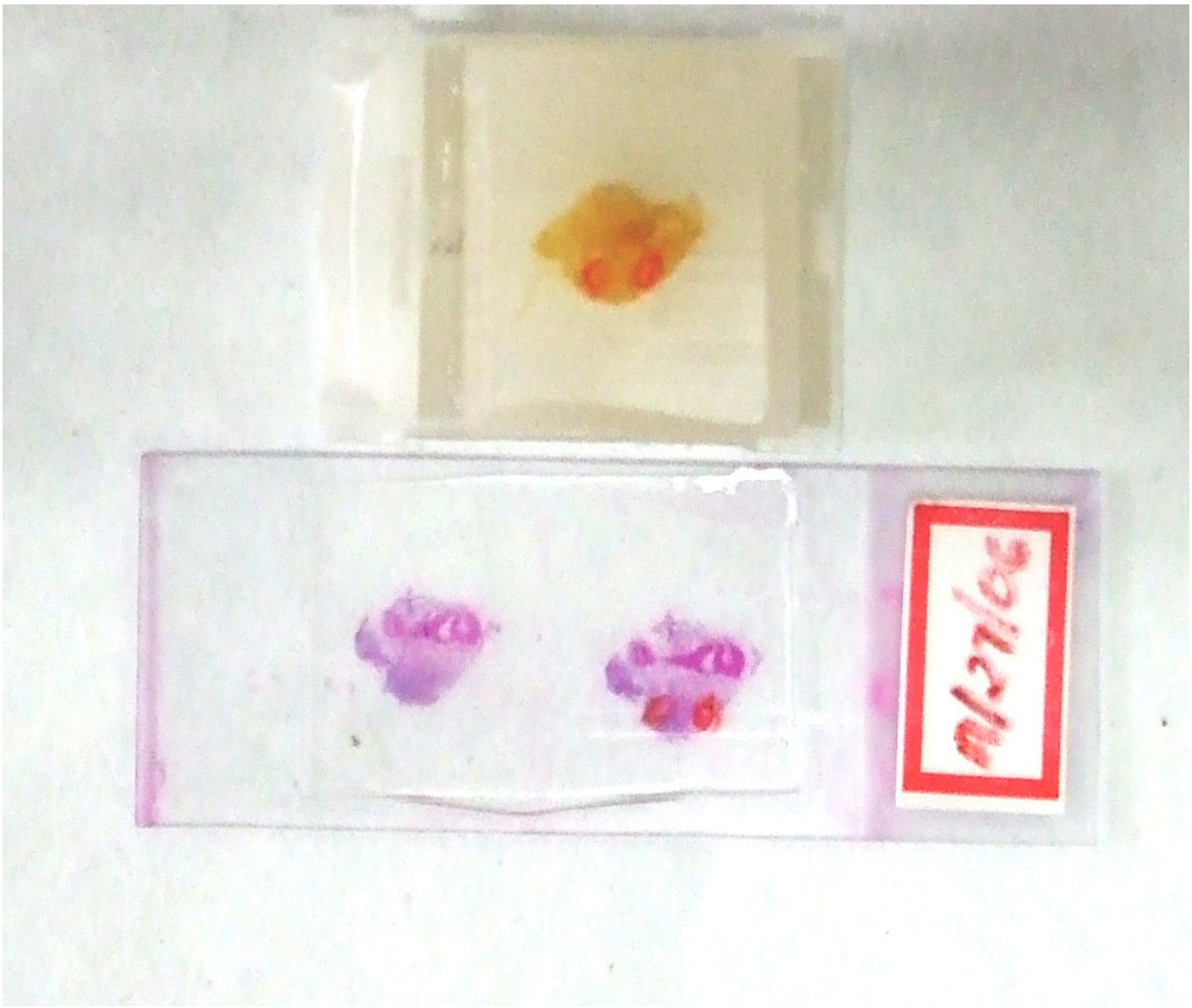

Figure-5
Identifying and mapping the representative areas of the tumor on tumor specimen in the paraffin block

Manual Tissue microarrayer

The recipient block is secured to the immobile stage holder at the base of the microarrayer. First a small hole is made on the recipient block on the stage using the left-hand hollow stainless steel punch of the mobile turrent. Next the donor block is placed on a custom- built small bridge over the stage that holds the recipient block. The right- hand punch with a core diameter of 0.6 mm is positioned above the representative area on the donor block and is moved into the punch position. Now the core is collected in the hollow stainless steel tube. A solid stainless steel wire, which is closely fit in the tube acts a stylet, it is gently pushed to extrude the core from the hollow punch. The core is examined and then carefully inserted into the hole in the recipient block. An adjustable depth stop maintains the length of the recipient holes and the transferred core to 3-4 mms. The punch unit is moved to a new position using the precision guide. The precision guide allows the needle to move only parallel to its own axis either along the X axis or Y-axis. The distance moved is displayed on digital micrometers hence the distance between the cores can be uniform and optimum of 1mm distance should be maintained [3]. The sampling procedure is repeated to punch different regions in the same block as well as from the other donor blocks and cores are precisely arrayed on the recipient block. As the cores are arrayed on the recipient block their identification details are recorded on the Excel sheet (figure 6). The TMA block is now treated as a regular paraffin block and multiple sections can be cut from this Tissue microarray block.

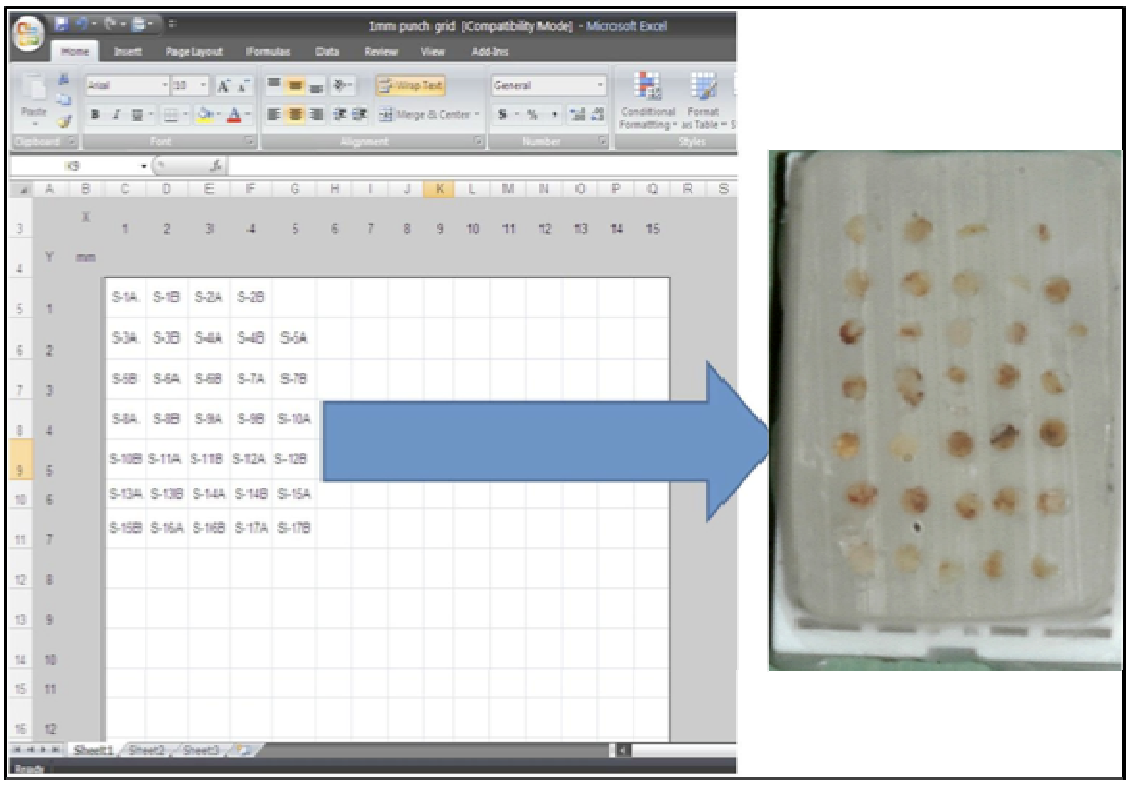

Figure-6
Representing the location of the cores in the TMA Block on an excel sheet

Automated tissue microarrayer

The automated arrayeris capable of making multiple replicate tissue microarray blocks from a single set of donor specimens. The automated tissue arrayer is connected to a monocular microscope and a computerized program[8]. The donor blocks and recipient blocks are fixed in their slits in the arrayer and their positions enter on the software program. The image of the tumor specimen in donor block is acquired on the monitor and its corresponding tissue section is placed on the viewer of the monocular microscope. The histopathological image is superimposed on the image of the tumor specimen and the most representative tumor areas are identified and marked. Once all the tumor areas in all the donor blocks are identified and the identification details of each donor block uploaded. The program is run and the arraying procedure commences. At one run 27 donors and 3 recipients blocks can be placed in ATA-27. The donor blocks can be replenished and new ones loaded throughout the TMA construction. It takes about 4 hours for the punching and choosing of spots on the blocks to make one recipient array. It is possible to obtain tissue sample from upto 20 sites without causing much damage to the original block.

Benefits of tissue microarray technology[9-16]: i. The main tumor block can be preserved and used for verification of clinical diagnoses and for future researchas only small cores of tumor tissue are taken from the tumor block. ii. All the tumor samples are subjected to the same working procedure. The reagent concentrations, the incubation time, temperature, wash conditions and antigen retrieval are all identical for all the cases. iii. Since a large number of specimens can be analysed simultaneously, various statistical tests can be applied and more accurate results can be obtained. iv. There is a substantial reduction in the number of sections that need to be cut. There is just a single slide that needs to be stained. For example, it: would take 6-8 hrs to construct a TMA of 240 cores, cut and stain sections of this tissue microarray. While, it would take a day to cut and stain the same 80 cases if the conventional one-slide one section method is followed, vi. There is minimal damage to the donor and recipient block .The morphological details in tumor cores were well preserved. vi. Tissue microarrays can be constructed in a cost-effective manner, vii. The expensive reagents such as the primary antibodies, conjugates and chromogen are economizedand only a few µl of reagent is necessary to analyze the entire cohort. viii. There is a 10,000 fold amplification of limited resources: from one tissue biopsy, the microarray technique can produce material for 500,000 assays. ix. The technology enables quality control and standardization of staining procedure including interpretation of the results. x. The ability to use archival specimens in high throughput molecular analyses is a significant advantage. xi. The speed of molecular analyses is increased by 100- fold, precious tissues are not destroyed and a very large number of molecular targets can be analysed in consecutive sections.

Limitations: For optimal preparation, we have to ensure that the cores obtained contain the most relevant and representative tumor areas [11].It is of utmost importance to identify the representative regions of the tumors in the donor tumor samples. The surface area of each sample of a 0.6mm tissue core is 0.282 mm^2, or in pathologist terms about the size of 2-3 high power fields, whereas 2X2.5mm of tissue area is available in whole sections. Hence, precise localization of tumor areas has to be defined to obtain meaning information. At times, multiple samples have to be collected from different sites in each tumor[2].

With several hundreds of cores in a single block it is vital to keep a detailed map of each core's position. A particular tissue can be used as a fixed landmark and keep proper maps spreadsheets and hard copies[16].

Another problem with TMA is the loss of 15% of cores between arraying and the final slide. This happens because cores are not at the level of the surface of the wax blocks. The cores may dislodge from the glass slides during processing[16].

Size of the donor cores: The current microarrayers carry donor puncher needles of core diameter of 0.6 mm, 1 mm, 2 mm or 4 mms. Using larger needles causes more damage to the original tissue blocks and substantially reduces the number of specimens that can be arrayed. For example, only about 100–150 cores measuring 2 mm in diameter can be placed in a single TMA block[2,3]. The core with a diameter of 2 mm contains ten-fold larger area for observation than the core with a diameter of 0.6 mm[9,14] (figure 7 and figure 8). Larger areas enable the researchers to easily discriminate cancer cells from non-neoplastic cells. In contrast, reducing the needle size to 0.4 mm could enable construction of arrays with 2500 specimens in a single TMA block. In the previous reports, the core with a diameter of 0.6 mm was frequently used. A balance has to be maintained, an optimal number of cases that can be analysed simultaneously as well as there should be sufficient tumor material to obtain information on the cellular characteristics of the neoplastic cells[2].

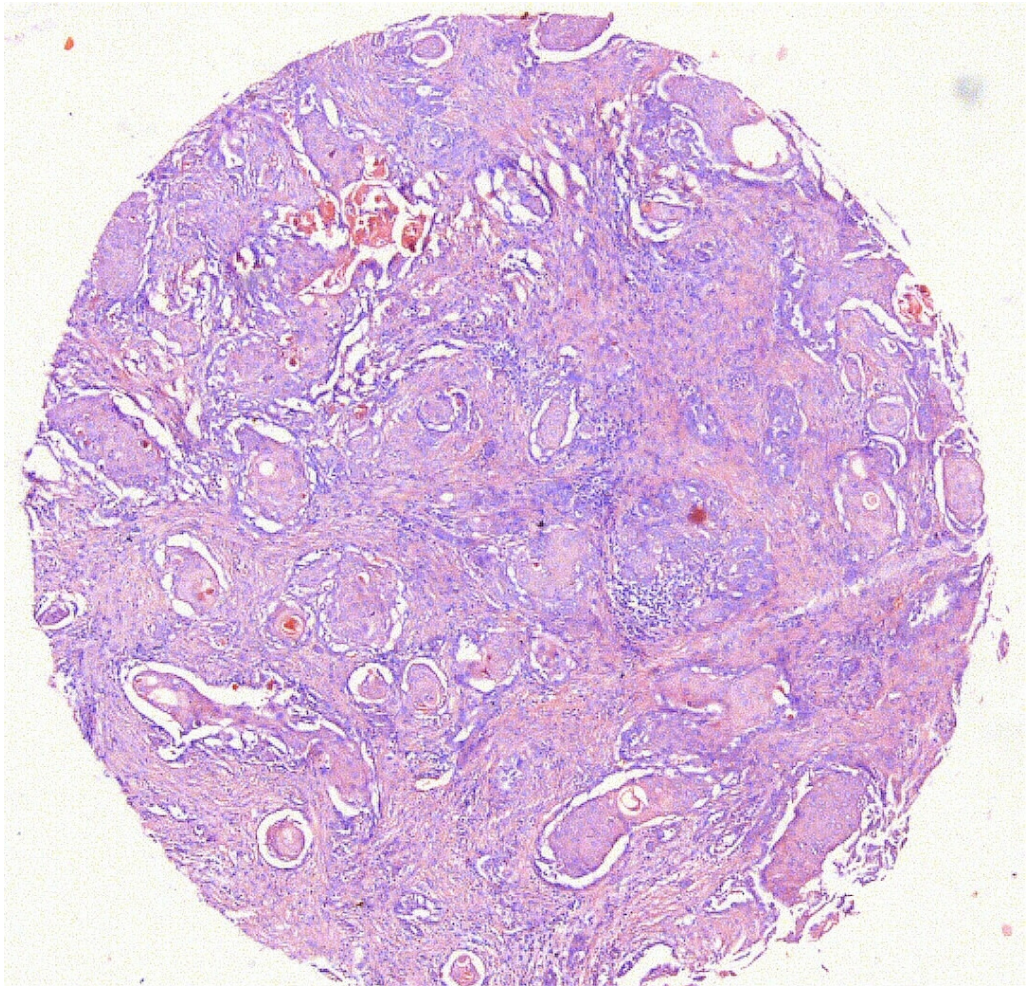

Figure-7
2 mm core of Oral Squamous cell carcinoma (H& E 10X)

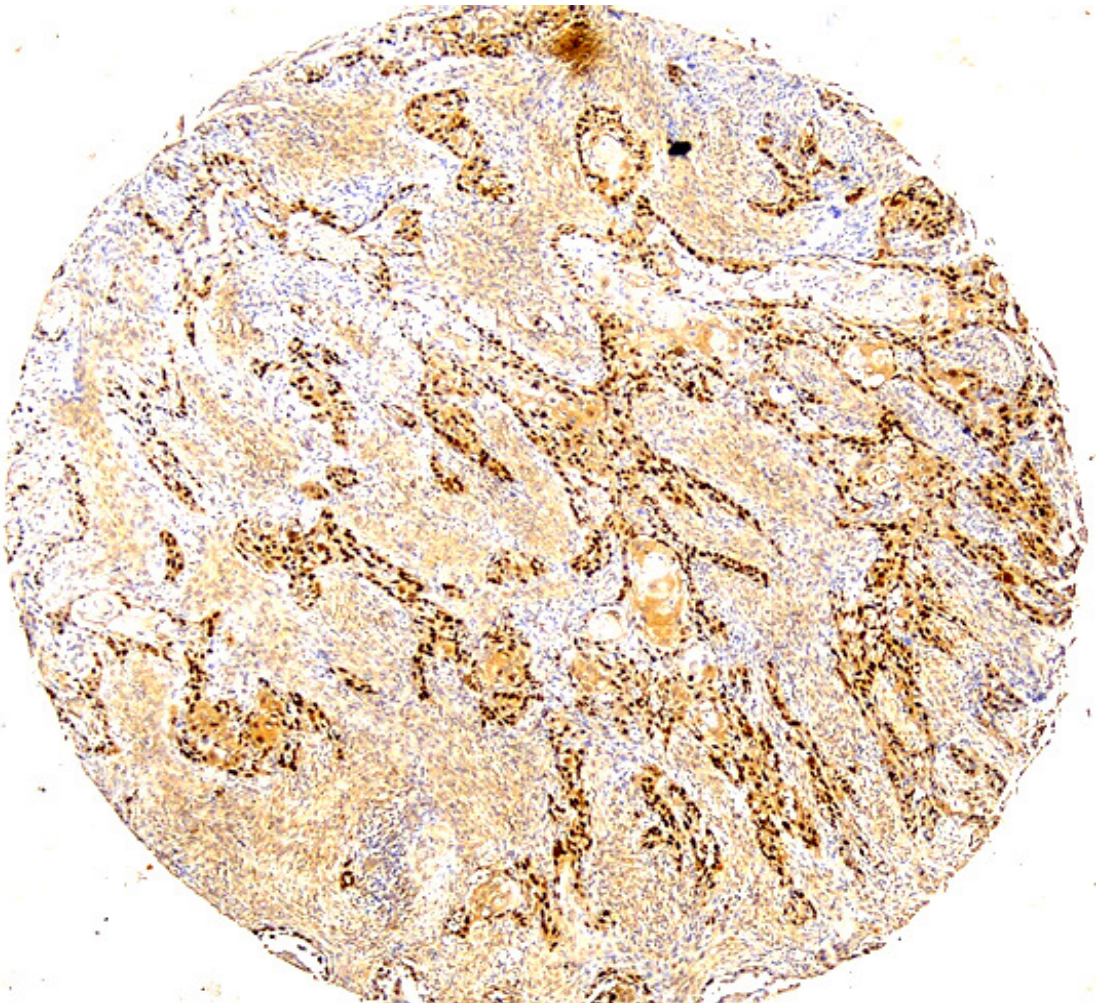

Figure-8
2mm core of Oral Squamous cell carcinoma – (IHC p53 10x)

With a configuration of 0.6mm diameter tissue cores arranged with a spacing of 0.8 mm, the maximum number of samples that can be arrayed in a 45 x 25 mm paraffin block is about 1000, but usually 400–800 specimens are arrayed per TMA block[3].

TMA and Whole Sections: Many studies have been carried out to test the validity of tumor cores to reliably represent the protein expression as in whole sections[15,16]. Monterio LS et al 20103 demonstrate that dual 1.5 mm core TMA is a valid, rapid, economical and tissue-saving way to study OSCC biopsies and that it presents strong correlation with the whole sections[17].

Application of Tissue Microarray in oral squamous cell carcinoma

With its wide spread use Tissue microarray technology has developed into a remarkable invention in cancer research. The most common approaches for TMA technology applications are in detecting protein antigens using immunohistochemistry (IHC) and for identifying gene amplifications using fluorescent in situ hybridization (FISH)[2]. The TMA is considered as a population level research tool for cancer. The blend of TMA with IHC is a powerful approach to detect molecular changes taking place during the pathway of carcinogenesis. The various studies in which TMA microarrays were used in combination with immunohistochemistry for oral cancer research is given in table 1.

There are several types of tissue microarrays that can be designed to carry out research in oral cancer.

Tumor Progression arrays: These Tissue Microarrays comprise of a range of tissues that include normal, premalignant and malignant oral lesions. Morphological and molecular changes that take place through the progression of the lesioncan be assessed[24].

Prognostic arrays: The Tissue Mircoarrays consists of several tumor samples in which any change in the protein expression is identified. The alteration is correlated with clinic pathological

parameters and follows up details such as recurrence, survival rate and metastasis[5,29].

Arrays for multiple marker analysis: Tissue microarrays are constructed with several tumor samples. Sections cut from these blocks are tested for the expression of a multiple markers. Thus an entire cohort is analysed simultaneously and rapidly [5,21,25,26,29].

Conclusion

The tissue microarray technology can be mounted to two significant features, firstly, numerous tumor specimens can be analysed at once and second, the tissue samples can be evaluated for numerous molecular markers simultaneously. The molecular characteristics individual tumors can be of the tumorcan be relatedto habits, site, stage of the tumor and the grade of the tumor (figure 9).

Table-1
Scientific studies that used a combined tissue microarray and Immunohistochemistry approach for Oral Squamous Cell Carcinomas

Authors	Sample Size	Biomarkers used	End Point
Lopez de Cicco et al 2002 [18]	*118*	Furin	Validation, multitumor analysis and tumor progression
Freier et al 2003[19]	*547*	Cyclin D1, c-myc, erbb 1 and erbb2	Correlation with clinic-pathological data
Marcus et al 2004[20]	*102*	CD68	Correlation with clinic-pathological data and patient survival
Fillies et al 2005[21]	*85*	Catenins	Prognostic Indicator
Fillies et al 2006[22]	*308*	Cytokeratins and Vimentin	Correlation with clinic-pathological data
Kleer et al 2006[23]	*113*	Rho C GTpase	Prognostic Indicator
Garcia et al [24]	*124*	E Cadherin, Laminin and Collagen 1V	Tumor progression assessment
Laimer et al 2007[25]	*109*	EGFR 1	Prognostic Indicator
Karsai et al 2007[26]	*664*	p16, p53	Frequency of expression
Solomon MC et al 2010[5]	*30*	p53, Bcl-2, E- Cadherin	Frequency of marker expression.
Monterio LS et al 2010[17]	*39*	EGFR, Ki-67	Candidates for EGFR targeted therapy
Coutinho- Carmillio et al 2011[27]	*229*	Caspases	Caspases are important in tumorigenesis
Moar et al 2012[28]	*50*	Bcl 10	Not a Prognostic indicator
Solomon MC et al 2014 [29]	*161*	Cyclin D1 and BCl-2	Correlating the expression of the markers with each other and with clinic-pathological features

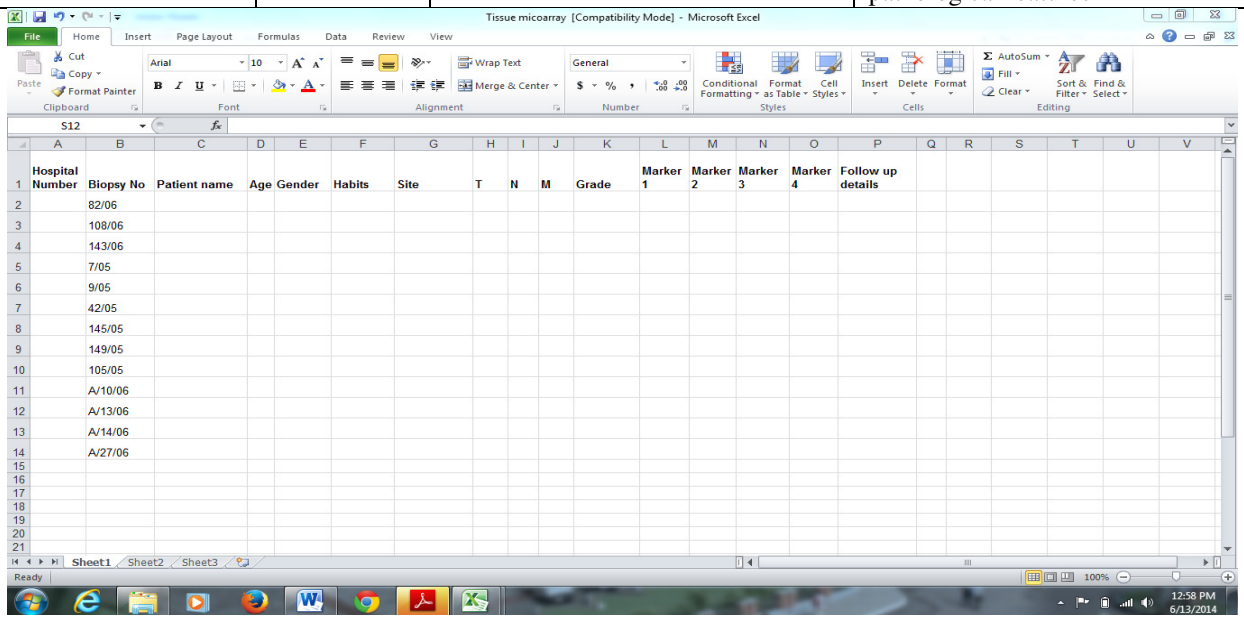

Figure-9
Linking the molecular data of individual cases with clinico –pathological features and follow-up data

The additional molecular data obtained through TMA will help in recognizing patients who are prone to develop a recurrent tumor or those who will not respond well to conventional treatment protocols. Thus, with a better insight into the biological aggressiveness of the tumors, personalized treatment protocols can be defined. This will have a tremendous impact on the quality of life of the cancer patient. Further, tissue microarray technology which is regarded as a population level research tool can be translated into aneffective investigational tool.

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