USE OF TISSUE MICROARRAYS IN TRANSLATIONAL RESEARCH

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ABSTRACT: *Tissue microarrays (TMA) technology has been widely developed in the last years to perform high-throughput immunohistochemical analyses for all tissue-based oncology research. TMA has completely revolutionized biomedical research, mainly to identify prognostic and predictive biomarkers. Many cores of tumour from paraffin-fixed tissue are incorporated into a single paraffin block to obtain a single slide used to perform simultaneous analysis of molecular targets. Recently the use of TMA has been implemented in clinical settings, in order to provide cancer specific molecular data, needed to define the correct therapeutic strategy. The aim of this review was to analyse the fields of application of TMA technology in oncology, emphasizing the adequacy/inadequacy for the immunohistochemical detection of cancer stem cells (CSCs) in human tumours and to test the feasibility of TMA for investigation of biomarkers within the tumour microenvironments.*

KEY WORDS: *Tissue microarray, Immunohistochemical analyses, Cancer biomarkers.*

INTRODUCTION

TMA technology, described by Kononen et al[1] in 1998, revolutionized the investigation of potential cancer biomarkers. TMA is an array-based high-throughput technology used to examine molecular alterations in a large number of tissues. In this view, the concept of DNA microarrays was extended to embedded tissue samples of pathology archives[2]. It has been widely used for tissue-based research, particularly when using immunohistochemistry and In Situ Hybridization (ISH) technique[2]. TMA should allow an efficient and rapid analysis of large tissue numbers, whereby the representatively and the adequacy of the core for the corresponding full-section slide is crucial for this technique. Many advantages are recognized in TMA experiments with respect to experiments driven of whole sections from different samples, mainly for the possibility to analyse different markers in the same area previously selected and reducing variability linked to pre-analitic conditions[3].

Candidate genes identified in other high-throughput technologies, such as serial analysis gene expression and array comparative genomics hybridization, were frequently validated in TMA studies[4], and in order to review the technique on different tissues, several validations studies with different types of cancers have been performed in recent years.

Clinical diagnostic use of TMAs is limited due to the limited sample size; however, they have been used for quality assurance purposes in the clinical setting, such as inter and intra-laboratory concordance[5].

USE OF TMA FOR RESEARCH LABORATORY

TMAs can be used to study tissue morphology, expression of proteins or genes and chromosomal aberrations using different stains, immunohistochemistry and in situ hybridization[3-5] (Figure 1). It is calculated that a TMA block of 300 cores
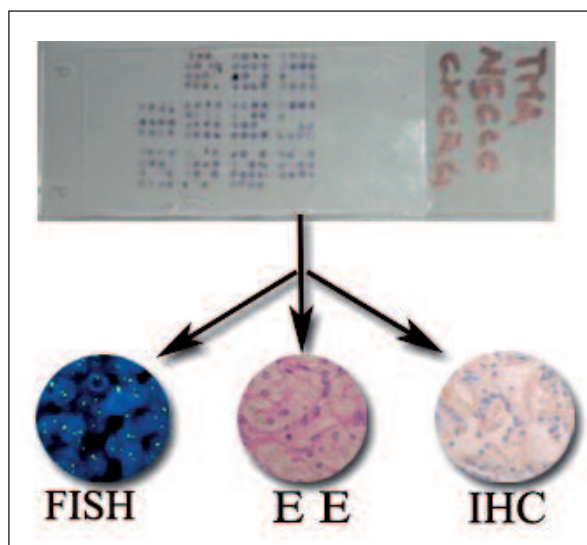
Figure 1. Use of TMA. TMAs can be used to study tissue morphology, expression of proteins and chromosomal aberrations using different stains, immunohistochemistry and in situ hybridization.

would produce 375,000 different results[1-5]. An accurate selection of the significant areas in the block donors is crucial for TMA design. Thus, selected tissue cores, from 0.6 to 2.0 mm in diameters, from formalin-fixed paraffin-embedded (FFPE) are included into an empty paraffin block, defined in x-y position (Figure 2). Different TMA-based research strategies have been proposed in cancer research. In particular, multitumour, progression and prognostic TMA have been widely used. Multitumour TMA are used in qualitative and quantitative screening of the distribution of specific biomarkers in various tumours[6-8]. A study[9] reported the different tumoural expression of HOXD13 from corresponding normal tissue of origin, showing a reduction of expression in gastric and pancreatic cancers. On the contrary HOXC13 increased expression seems to be related to melanoma progression[10]. Progression TMAs are used to define morphological and molecular alteration in tumour progression. Its application has been particularly used in the study of hepatocarcinoma and prostatic cancer[1,11,12]. The most common application of the TMA technology is in the field of retrospective studies, through the use of Prognostic TMA, which is commonly used to identify markers responsible for tumour progression and therefore correlated to a worse prognosis. It is associated with a database containing all the follow-up data of the patient. EZH2 was related to outcome after radical prostatectomy, being strong expression related to disease progression[11]. HOXB13 expression is related to poor prognosis superficial transitional cancer[13]. Finally, prognostic TMA could be used also to build a mathematical predictive model, analysing different biomarkers in the same prognostic series[14-16].

Therapy-based TMA are currently used to define the responsiveness to specific target biotherapy and to classic chemotherapeutic strategies. Particularly TMA-based studies have provided useful information about expression of target therapy in tumour different from those in which they firstly have been found, such as HER2 amplification in a subset of gastric cancer[17]. In addition, in retrospective series with homogeneous chemotherapeutic treatment, TMA could be used to identify biomarkers related to poor response. Finally, TMA for research purpose can be built, using biopsies, frozen tissue and cell lines[2-5].
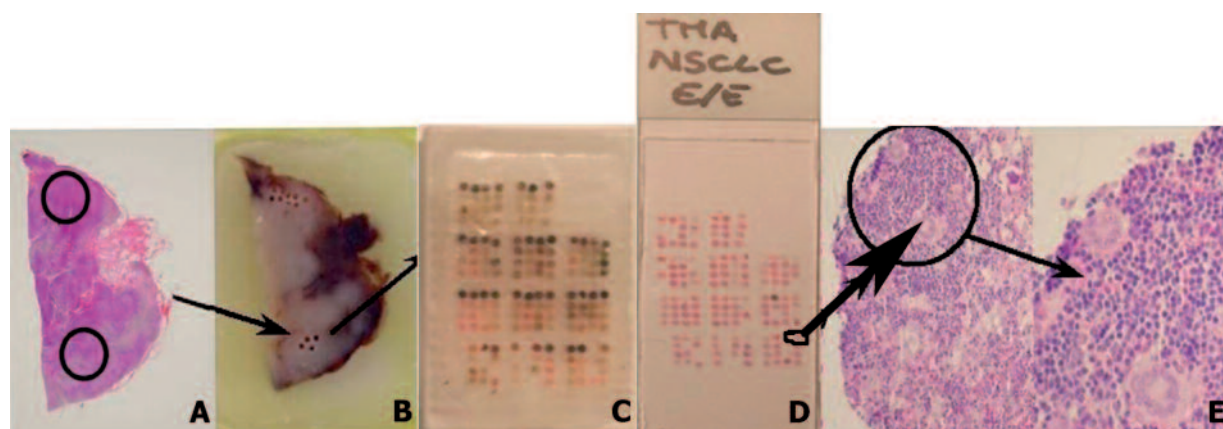

Figure 2. TMA construction step. **A,** Tissue microarray was built using two cores from different areas (a superficial one and one representative of the deep invasion). **B,** Tissue cylinders were punched from morphologically representative tissue areas of each "donor" tissue block. **C,** Tissue cylinders were brought into one recipient paraffin block (3 × 2.5 cm) using a semiautomated tissue arrayer (Galileo TMA). **D,** H&E staining of a 4 ☐m TMA section was used to verify all samples. **E,** Every case were reviewed by experienced pathologists.

BENEFIT AND PITFALLS OF TMA TECHNOLOGY

TMAs provide a fast workflow for the evaluation of biomarkers in a series of patients, in a unique experimental approach. The possibility of using small amounts of tissue blocks for building a specific TMA led to biomaterial saving, favouring multiple studies on the blocks[1-5]. The combination of TMAs and clinically annotated samples represents an elegant and cost-effective approach to study panels of expression under identical experimental conditions and to develop prognostic or predictive models of patient outcomes.

Despite these considerable advantages, TMA technology is not without limitations. Pitfalls in TMA studies are mainly related to technical, interpretative and statistical features[18,19].

Firstly TMA sections felt all effects of not good tissue pre-analytic preservation observed in the whole section. Thus validated antibodies and standardized techniques could not provide expected results, above all when donor tissues originated from different institutions. Moreover, as whole histological section, TMA sections could be altered by oxidative effects responsible of loss quality of immunostaining. Preservation of antigenicity could be obtained storing paraffin coat slides in a nitrogen desiccator[2].

Tumour heterogeneity can have a significant impact on the interpretation of biomarkers, so is required the use of more spots for each one case. Generally the inclusion of two cores per case provides immunostained percentage of cells comparable to conventional tissue section[20,21].

Finally, many statistical analyses are currently used to evaluate the association of the tested biomarkers with other patients' clinic-pathological data and survival. The choice of the critical cut-off for novel biomarkers remains often arbitrary, but the use of sophisticated biostatistical algorithms could solve the definition of the optimal cut-off[2].

USE OF TMAS IN CLINICAL LABORATORY

Clinical use of TMAs is restricted to assay development and validation and also for quality control test but not yet to provide a specific diagnosis. TMA can be used for assay development and validation, as initial tools to optimize and validate clinically relevant assays. Indeed, a new antibody could be tested in multiple assay conditions on a serial sections of the same TMA block, including a range of differentially expressing samples[5]. Thus, a dynamic range can be calculated whereby the high-level expressing cases are compared with the low level or negative-expressing cases[5].

The TMAs are also used for quality assurance to assess intra- and inter-laboratory assay reproducibility. The College of American Pathologists is increasing the use of TMAs for laboratory proficiency testing[22]. An interlaboratory quality assurance study of HER2 overexpression and relative gene amplification, through the use of TMA including cores from 80 breast cancers, revealed 70% of the samples had 90% concordance among 243 laboratories[23]. TMA could be used for the diagnosis, specifically for breast cancer immunohistochemical profile. Routine diagnostics of breast tumours includes histological typing, grading, pathological staging as well as profiling by use of an immunohistochemistry panel of antibodies, including steroids receptors, HER2 and ki67 and in situ hybridization, related to HER2 gene amplification[6,7]. Multi-core tissue micro arrays including cores from routine blocks of breast cancers may be used for definition immunohistochemical profile and HER2 amplification in routine work. Results from routine TMAs completely overlapped with results on whole section for each one case in a series of 234 patients[6]. Recently it has been demonstrated that 2 mm breast tumour cores correlate with the corresponding tumour on whole mount slides, regarding staining/hybridizing results with the biomarkers, including also Topoisomerase II and EGFR amplification[7].

USE OF TMA FOR CANCER STEM CELLS DETECTION IN HUMAN TUMOURS

Recently, TMA technology has been extended to characterization of cancer stem cells (CSCs) populations within tumor microenvironment. An IHC assay was performed for CD44 detection on TMA of gastric adenocarcinoma, showing an increased expression in intestinal metaplasia[24]. A TMA was used for CD44/CD24 detection in pancreatic cancer[25]. ALDH1 expression was detected on breast cancer TMA, where its prognostic role is correlated to patient's age and on pancreatic cancer TMA where it represents an important prognostic marker as reported by a study[26].

The same marker on triple negative/basal-like breast cancer TMA has shown an aberrant expression in the stromal component of the tumours and it has been associated with a better prognosis[27].

Again, CD133 and CD44 markers were detected in paediatric solid tumours through TMA employment, highlighting their potential prognostic clinic value[28].

There are few studies about the validation of TMA technology for CSCs. In a recent study[18] has
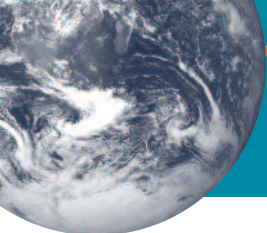
been shown that there are significant differences between TMA cores and full section slides. They compared the expression of the three CSCs markers (CD133, ALDH1 and CD44) on TMA cores and on whole sections of the same samples. This has allowed them to re-evaluate, in most of the cases, the expression of the markers assigning values to the percentage of positive cells which were completely different from those values assigned to TMA cores. Moreover, some cases proved positive where the spots were originally negative for CSCs markers. Therefore, the employment of a specific breast cancer TMA, showed a non-perfect correlation with the expression analysis carried out for the same markers on the corresponding whole section. The study shows some limitations for the immunohistochemical detection of CSCs in human tumours using TMA technology[18].

USE OF TMA IN TUMOUR MICROENVIRONMENT INVESTIGATION.

TMA technology could be extend in tumour microenvironment investigation. Indeed, the characterization of the elements involved in cancer immune regulation needs the analysis of several markers, the use of a TMA containing core of tissue that adequately represent the inflammatory infiltrates (TIL) in the intra-tumoural and peri-tumoural area could be an useful tool for the study of immune system. Zlobec et al[29] showed that new generation TMA (ngTMA) is an ideal platform to study of immune markers CD3, CD8 and CD45RO which are proposed as important prognostic immune infiltrates in the literature. In another validation study Utilizing CD3, CD8, CD20, CD45, FOXP3 and Granzyme B specific antibodies as markers has been performed immunohistochemical analysis on both TMA and matched whole tissue sections with the aim to determinate the concordance of expression of markers between TMA cores and whole sections. Specifically, in peri-tumoural areas the CD3 cells (highly expressed) in whole tissue section is found to have a concordance rate of 95% compare to corresponding 4 spots of tissue in TMA, while expression of Granzyma B cells (poorly expressed) reaches 80%. Instead, in the intra-tumoral area the CD3 cells in whole tissue section is found to have a concordance rate of 83% compare to corresponding 4 spots of tissue in TMA while the FOXP3 reaches 63%. In conclusion, the dates demonstrate that the TMA can be considered a useful tool for the study of most representative immune cell population (CD3, CD8, CD45RO) in RCC tumour microenvironment but not for poorly representative immune cells (FOXP3, Granzyme B)[19].

CONCLUSIONS

High through output molecular screenings have provided information about the role of many markers in cancer pathogenesis and development. TMA technology has offered a powerful method to validate these biomarkers in order to define their real impact in the neoplastic progression. Thus, TMAs represent useful tools to identify effective biomarkers, both for prognostic-predictive purposes and therapeutic strategies. TMA technology can be used for high throughput marker trial in order to save tissue, time and effort and to review many markers on numerous cases to select the markers with the most promising results to use them on the full sections for more differentiated results. TMA should allow an efficient and rapid analysis of large tissue numbers, whereby the representatively of the core for the corresponding full-section slide is crucial for this technique.

One major problem of taking samples of full-section tumour blocks is the heterogeneity of marker expression of the tumour cells, which is predicted to show a unique expression pattern for each marker. The studies of comparison and validation of TMA both in the field of stem cell markers that the immune system have shown that the TMA technology is not a good tool for the study of cellular populations poorly represented within the tumor. In conclusion, TMA technology could be recommended to be used with markers of a high expression and a more homogeneous staining pattern. Therefore, for markers expressed in few cells which show a focal and heterogeneous staining pattern, the full section slides is currently the superior method.

CONFLICT OF INTERESTS:
The Authors declare that they have no conflict of interests.

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