The use of tissue microarrays in oncology: from research to diagnostic application

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Abstract
Tissue microarray (TMA) technology has been widely developed and utilized in recent years for all tissue-based oncology research, mainly to identify prognostic and predictive biomarkers in cancer research. Recently the use of TMA has been implemented in the clinical setting, in order to provide cancer-specific molecular data needed to define the correct therapeutic strategy. In this context, the definition of the receptor status of breast cancer seems to be the best example of application in clinical pathology laboratories. In this review, we analyze the fields of application of TMA technology in oncology.

Key words: diagnosis, oncology, tissue microarrays

Introduction

The use of genomics and proteomics for biomarker identification for new diagnostic and therapeutic applications has greatly increased, leading to the development of high-throughput technologies. In view of this, the concept of DNA microarrays was extended to embedded tissue samples from pathology archives [1]. Tissue microarray (TMA) technology, first described by Kononen et al. [2], is an array-based, high-throughput technology used to examine molecular alterations in a large number of tissues on parallel slides [2]. It has been widely used for tissue-based research, particularly in immunohistochemistry and in fluorescence in-situ hybridization (FISH) techniques [1]. Many advantages of TMA are recognized with respect to experiments using whole sections from different samples, mainly because of the possibility of analysing different markers in previously selected areas, and reducing the variability linked to pre-analytic conditions [1-3]. As the number of cancer studies using high-throughput technologies increases, TMA technology is a proven high-throughput tool for validation of marker genes identified in DNA microarray experiments. In fact, candidate genes identified in other high-throughput technologies, such as serial analysis gene expression and array comparative genomics hybridization, have been frequently validated in TMA studies [4].

Clinical diagnostic use of TMAs is constrained due to the limited sample size; however, TMAs have been used for quality assurance purposes in the clinical setting, such as inter- and intra-laboratory concordance [5]. In addition, the extensive use of immunohistochemical and FISH analysis in breast cancer for definition of therapeutic strategies led to the use of TMA in routine samples in some hospitals with wide experience in breast cancer diagnosis [6, 7].

Finally, the use of TMA has been proposed for the selection of tumour areas most likely to be helpful for molecular diagnosis, through nucleic acid extraction [8].

Use of TMA for research laboratory

TMA needs to be designed so that it can accurately select the significant areas in the block donors. Thus selected tissue cores with diameter from 0.6 to 2.0 mm are included in a recipient paraffin block, defined in an x–y position. Currently, since most TMAs are being used for immunohistochemistry and ISH techniques, it is calculated that a TMA block of 300 cores would produce 375,000 different results [1-5].

Different TMA-based research strategies have been proposed in cancer research. In particular, multi-tumour progression and prognostic TMA have been widely used. The use of *multi-tumour TMA* promotes qualitative and quantitative distribution of specific biomarkers in a wide range of different tumours [1]. In our experience, the differentiation of HOXD13 tumour expression from normal tissue has been revealed in most tumours, but is significantly decreased in gastric and pancreatic cancers compared with normal tissue [9]. Conversely, increased HOXC13 expression seems to be related to melanoma progression [10].

Progression TMAs are used to define morphological and molecular changes through the different stages of

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tumour progression. Its application has been used particularly in the study of hepatocarcinoma and prostatic cancer [1, 10, 11].

The most common application of the TMA technology is in the field of retrospective studies, through the use of *Prognostic TMA*. It is generally associated with clinical follow-up data. Thus the significant role of a specific biomarker on patient prognosis could be easily revealed if the cohort of patients is broad enough. The development of TMA in retrospective studies has greatly increased the number of potential biomarkers related to prognosis [1-5]. For example EZH2 expression was found to be related to outcome after radical prostatectomy, with strong expression related to disease progression [11] and HOXB13 expression is related to poor prognosis superficial transitional cancer [12]. Furthermore, some biomarkers potentially related to targeted therapeutic agents seem to be related to worse prognosis, such as phosphorylated epidermal growth factor receptor (EGFR) in oral and penis squamous cancer, independently from classical activating mutations observed in lung adenocarcinoma, thus opening interesting new perspectives in the treatment of these specific neoplasias [13-15]. Additionally FISH analyses have been conducted on prognostic series, but a small series of prognostic chromosomal aberrations have been produced.

Interestingly, cyclin E gene amplification and relative protein overexpression directly correlate with bladder cancer outcome [16]. Finally, prognostic TMA could be used also to build a mathematical predictive model, analysing different biomarkers in the same prognostic series [17-19].

Therapy-based TMAs are currently used to define the responsiveness to specific target biotherapies and to classic chemotherapeutic strategies. In particular, TMA-based studies have provided useful information about expression of therapy targets in tumours different from those in which they firstly have been found, such as HER2 expression amplification in a subset of gastric cancer [20]. In addition, in retrospective series with homogeneous chemotherapeutic treatment, TMA could be used to identify biomarkers related to poor response. Indeed TMA of cases of cervical cancer with high and low chemoradiotherapy responsiveness showed that high expression of S100A9 and galectin-7 with low expression of NMP-238 and HSP-70 was related to high responsiveness to chemoradiotherapy [21]. Finally, TMA for research purpose can be developed using biopsies, frozen tissue and cell lines [1-5].

Benefit of TMA studies

TMAs provide a fast workflow for the evaluation of biomarkers in series of patients in a unique experimental approach. Furthermore, theoretically, the role of the pathologist is restricted to the selection of the areas from donor blocks, while interpretation could be performed by non-experts with only a rudimentary training or even by machine. In addition, TMA has led to significant cost savings respect to whole section analysis, since a single unique experiment allows a large cohort of patients evaluation. Finally, the possibility of using small amounts of tissue blocks for developing a specific TMA has led to substantial savings in terms of biomaterials, favouring multiple studies from the same tissue blocks [1-5].

Pitfalls of TMA technology

Pitfalls in TMA studies are mainly related to technical, interpretative and statistical features [1-5].

Technical features

The quality of TMA sections is affected by effects of poor pre-analytic tissue preservation in the whole section. Therefore, validated antibodies and standardized techniques cannot provide the expected results, particularly when donor tissues have originated from different institutions. Moreover, like whole histological sections, TMA sections could be altered by oxidative effects resulting in loss of quality of immunostaining. To avoid this problem, antigenicity can be preserved by storing paraffin coated slides in a nitrogen desiccator [1].

Interpretative features

Tumour heterogeneity could be responsible for unequal antigen distribution. Thus, heterogeneous biomarker distribution requires the use of more spots for each case. Generally, the inclusion of two cores per case provides a proportion of cells that can be immunostained and which can be superimposed on the conventional tissue section [1-5]. Indeed, in Hodgkin's lymphoma – considered a prototypic heterogeneous neoplasm – a concordance rate of almost 95% between standard sections and respective TMA, including only one core, has been observed for CD20 immunostaining [22]. In other haematological malignancies the comparison between TMA cores and the corresponding whole section immunohistochemical and FISH data did not show any significant differences [23, 24]. Finally, experiences of tumours with different histological features, such as non-seminomatous tumours, have been reported in the literature [25].

Both manual and automated interpretation of the data is currently used. Automation seems to provide particularly reliable results for fluorescent data, while it appears more difficult to apply automated methods for immunohistochemistry identification of novel biomarkers [1-5].

Statistical analysis

Many different statistical analyses are currently used to evaluate the association of the tested biomarkers with other patients' clinic-pathological data and survival, commonly using continuous scoring counts. The choice of the critical cut-off for novel biomarkers often remains arbitrary, but the use of sophisticated biostatistical data analysis could solve the definition of the optimal cut-off [1].

Use of TMAs in clinical laboratory

Clinical use of TMAs will occur in limited settings, related to assay development, quality control and specific diagnoses. 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 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]. 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 [26].

An inter-laboratory quality assurance study of HER2 overexpression and relative gene amplification through the use of TMA including cores from 80 breast cancers, revealed that 70% of the samples had a 90% concordance rate among 243 laboratories [27].

In our experience, we manage an Italian inter-laboratory quality control program for assessing ALK rearrangement in lung cancer through FISH analysis in a small series of cases included in a TMA. The inter-observer concordance was not so high; however, this is generally expected for FISH analysis.

Finally, TMA can be used for diagnosis, specifically for breast cancer immunohistochemical profiling. Routine diagnosis of breast tumours includes histological typing, grading, pathological staging as well as profiling by using an immunohistochemistry panel of antibodies, including steroid receptors, HER2 and ki67, and ISH for HER2 gene amplification [6, 7]. Multi-core TMAs including cores from routine blocks of breast cancers could be used for definition of the immunohistochemical profile and HER2 amplification in routine work. Results from routine TMAs were shown to completely overlap with results on whole sections for every case in a series of 234 patients [7]. Recently, it has been demonstrated that 2 mm breast tumour cores correlate with the corresponding tumour on whole mounted slides, regarding staining/hybridizing results with the biomarkers, including topoisomerase II and EGFR amplification [6]. By scanning and digitalization of the TMA slides, the results could be optimized, reducing further the time needed for interpretation. Clearly this purpose can be applied whenever the work burden is significantly high, in order to reduce costs and time [6].

Conclusion

High-throughput 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. Moreover, the analysis of multiple markers could generate mathematic model of progression with significant improvement in the management of cancer patients. Furthermore, the challenge of this technology is the possibility of identifying critical factors predictive of therapy response in retrospective studies. This opportunity has to be promoted and should require more cores from multiple centres. TMAs have been shown to accelerate the process of drug discovery, by validation of drug targets, determination of molecular epidemiology and development of diagnostic assays.

Finally, the use of TMAs in the clinical setting would guarantee the development of laboratory assays, control assurance and multi-molecular profiling of breast cancer, with time- and cost-saving benefits.

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