How to make tissue microarrays

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Abstract

It is now almost 20 years since tissue microarrays (TMAs) were first described by Kononen and colleagues. Today, this high throughput methodology has been widely adopted by investigators who require a cost-effective method to rapidly and simultaneously interrogate large numbers of samples, including those derived from large patient cohorts. Drawing on over 15 years of direct experience of TMA design, construction and analysis, we discuss a variety of TMA applications and provide a detailed description of TMA design, quality control and construction. Advantages and disadvantages are discussed and potential problems and practical solutions are highlighted.

Keywords construction; design; quality control; tissue microarray

Introduction

Tissue microarrays (TMAs) were first developed and described by Kononen et al.[1] in 1998. Almost 20 years later, TMAs remain a fast and cost-effective solution for multiplexed in situ tissue analysis, and represent a validated method of high throughput simultaneous analysis to investigate a variety of biomarkers.[2]

Currently, TMAs are principally used as a research tool for the investigation of putative prognostic and predictive molecular targets in human cancer tissues. However, TMAs have been utilised for a variety of other applications including for diagnostic staining quality control, inter-laboratory comparisons[3–8] and infectious disease studies.[9,10]

Collections of tissue organised in a 'multi-tissue sausage' were first described by Battifora in 1986 (reviewed by Chan et al. [11]). This basic approach was further expanded and developed by Wan et al.[12] who produced a library of paraffin embedded cores set within a 'straw' to determine the staining patterns of new monoclonal antibodies. These techniques were refined in 1998 when Kononen and collegues,[1] using the term 'tissue microarray' for the first time, described the technology we still use today. In this seminal paper, Kononen eloquently elaborated on the advantages of using TMAs, which allow the processing of up to 1000 tissue specimens in one section, over using a conventional 'full-face' tissue section.[1]

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Types of TMAs

TMAs are generally categorised by their material of origin. If constructed from paraffin embedded material, the term 'tissue microarray' is usually applied, although this is not universally applicable as tissue samples may also be arrayed into resin as the recipient block medium. The latter is required if very thin sections are needed. However, resin TMA construction is technically challenging, labour intensive and is generally regarded as only being suitable for specialised applications because of constructional complexities when compared to paraffin TMAs.[13] Interestingly, TMAs have also been constructed using frozen tissue samples (cryoarrays)[14–16] as well as embedding cell lines[17–20] in addition to standard cell blocks.[21]

As an alternative to using the material of origin as a basis for classification, TMAs can also be categorised according to their anticipated application. Some examples are listed below:

- Predictive TMAs, which are used to identify markers that predict response to therapy such as for example HER2[22]
- Control tissue TMAs, which are used to establish experimental protocols and also serve as external controls for diagnostic immunohistochemistry (IHC).
- TMAs for validation of markers discovered by extracted protein, DNA or RNA based studies,[23–25] (see review by Hewitt SM)[26]
- Prognostic TMAs for investigation of the relationship between staining results and clinical endpoints[27–29]
- Progression TMAs in which cores of a single tissue type derived from different stages of tumour development or different tumour grades. Thus, for example, a progression TMA for breast cancer would include normal breast, ductal carcinoma in situ, invasive tumour and metastatic deposit[30] or for colon cancer one would array normal colon, adenomas with both, low and high grade dysplasia as well as carcinomas.[31]

It is important to note that TMAs are not only used to characterise abnormal tissues, but can also be used to determine the presence and extent of expression of proteins in normal tissues.[32,33]

TMA design and construction

Tissue sampling

The initial task of TMA design should be regarded as one of the most important stages of TMA construction.

Apriori hypotheses or questions of interest should be defined in advance as this will impact on the sampling strategy used to interrogate the original tissue samples. In particular, if it is intended to compare spatial protein expression patterns between tumour centre and periphery, cores will clearly need to be sampled from the appropriate locations. If, conversely, the task is to characterise overall protein expression for a given marker in a given tissue, then the sampling approach is completely different. Sampling for the first task (comparing the spatial expression of protein between a tumour periphery and centre) could be regarded as a 'targeted' approach whereas 'random' sampling would be the best technique to characterise an overall expression pattern in a tissue.[34]

Tumour heterogeneity is a recognised major challenge for TMA users.[35] Taking multiple samples of the tumour seem to be

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an appropriate method of compensating for potential variability of expression of molecular targets in a given tissue. Although there is no universally agreed standardised tissue sampling method, it is intuitive that the more samples are taken from the donor tissue of interest, the more representative the subsequent TMA staining results. Concerns relating to how representative TMA-derived staining data are when compared to full section staining has led to a large number of validation and feasibility studies in different tissues comparing results from whole sections with those of TMA cores [30, 34, 36–56]. How many TMA cores are necessary to achieve a high degree of concordance between results from full sections and TMA cores is a recurring question that is closely scrutinised. Most studies suggest that the results from triplicate TMA cores have up to 98% concordance with the result from full sections.[37,57] However, a recent study by Goethals et al.[58] recommend at least four cores whereas other authors achieved greater than 95% accuracy with only two cores.[56]

Beyond heterogeneity, basic technical issues mandate the use of more than one core of tissue per case. Tissue cores can be lost during sectioning and subsequent procedures, or subsequent interpretation is compromised by folding of the tissue core or unacceptably low numbers of tumour cells (assuming tumour cells are the component of interest) per core.[59–61] Thus by having multiple tissue cores per case, the potential impact of such loss is minimized.

The proportion of 'lost cases' resulting from technical losses has been reported as high as 23% in a TMA study of renal cell carcinomas.[62] We currently construct TMAs from gastro-oesophageal cancer specimens where we normally sample three 'random' cores from each area of interest. However, in the case of low tumour cell density which in particular is a problem in diffuse type gastric cancer, we often double the number of cores to six per case. Our own unpublished studies in gastric cancer TMAs suggest a mean technical loss rate of 10% of cores.

Interestingly, there is much less debate about the influence of core diameter with respect to tissue sampling. For the commonly used manual arrayer from Beecher Instruments (now manufactured by Estigen Tissue Science), punches with a diameter between 0.6 mm and 2 mm, equivalent to a tissue area of 0.283 mm^2–3.141 mm^2, are available. The vast majority of published studies use 0.6 mm punches, with cited benefits including a reduction in disruption to the donor block, preservation of more source tissue and incorporation of a larger number of cores in a single recipient block.

Up to 1000 cores of 0.6 mm diameter can be placed into a single TMA measuring 25 mm × 45 mm.[1,27] However, some authors are more cautious and suggest a maximum of 500 cores per block as a more realistic number[57]; this reflects our own ongoing practice. From our experience, punches with a diameter greater than 0.6 mm are useful in specific applications such as sampling of fatty or connective tissue-rich material as larger cores have better adherence. Also frozen tissue and study of large tissue areas e.g. whole depth of mucosa in the gastrointestinal tract require punches greater than 0.6 mm in diameter.

Layout of the TMA

Currently there is no general agreement on the optimal layout of a TMA, almost certainly because different studies have different requirements. From our own experience and through correspondence with other laboratories using TMAs, the following components appear to be essential to consider when planning a TMA layout.

As tissue border staining artefacts are a well-recognised problem when performing immunohistochemistry on full tissue sections, we frame all our TMAs with a 'protection wall' (Figure 1A), formed by a row of tissue cores which will not be analysed. Such protection walls are typically formed by any tissue that is available in abundance in the manufacturing laboratory. This protection wall was originally described by Hoos et al.[37]

Being able to unambiguously identify individual cores within the TMA section is crucial as any confusion or doubt about the origin of a core will make the assessment of the staining impossible. We recommend use of two separate features to ensure unambiguous orientation within the TMA section as well as unambiguous identification of the TMA block itself. Most authors add 'orientation cores' in specific positions usually outside the overall geometric margin of the array (Figure 1B). However, we were always concerned regarding loss of these crucial orientation cores and therefore incorporate orientation 'gaps' into the TMA design. Using a combination of intentionally left empty core positions, it is possible to unambiguously macroscopically identify the TMA block as well as to orientate the cut TMA section (Figure 1A). In addition, we include control tissue cores in every TMA and place them asymmetrically into the grid further aiding orientation within the section. Thus, control tissue cores serve as internal 'orientation cores' as well as both positive and negative internal experimental controls. We usually establish the staining pattern of the marker under investigation on sections from a control tissue TMA which contains the cores from the same control tissues as included in the final TMA.

The arrangement of TMA cores for a given design will depend on the type of study and on how many cores are sampled from each donor block. Ideally, cores from the same donor block should not be placed adjacent to each other as only a random distribution of cores from the same donor block within a given TMA would ensure results from individual cores are recognised as 'independent results' from a statistician's perspective.

However, from a practical perspective, randomly distributed TMA cores derived from the same donor block significantly increases the manufacturing workload and is therefore rarely done when using a manual TMA arrayer. Random distribution of cores is less of an issue if using an automatic TMA arrayer. We typically cluster cores from the same tumour and the matched normal tissue next to each other (Figure 1A) along the horizontal axis (from left to right). However, we recognise that other investigators or commercial suppliers of TMA sections use a completely different design (for example see Figure 1B). We would like to emphasise that our design presented in this paper is only one of many options for TMA design and individual investigators need to identify which methodology best suits their purpose.

Technical procedure

After appropriate cases of interest have been identified and tissue blocks retrieved from archive, a fresh full face 5 μm H&E section should be cut and reviewed using a conventional microscope or

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