High-density tissue microarrays from prostate needle biopsies

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ABSTRACT
Background Formalin-fixed prostate biopsies are frequently the only tissue collected at the time of prostate cancer diagnosis. There is therefore a requirement for techniques that allow the use of these prostate biopsy specimens in a high-throughput analysis of immunohistochemical and fluorescence-in-situ-hybridisation-detected biomarkers.

Methods The authors have previously described methods that allow tissue microarray (TMA) construction from prostate biopsies. Here, we describe significant technical innovations that provide an easier and more robust system of biopsy—TMA construction.

Results and discussion The TMAs produced are of a high density (up to 104 cores each, 8×13) and allow a multiplex analysis of biomarkers in the context of clinical trials.

TECHNICAL DEVELOPMENT
Prostate biopsy samples for these studies were collected from men with untreated, localised (clinical stage T1/2a, Gleason score ≤3+4; prostate-specific antigen <15; ≤50% positive cores) prostate cancer who were managed in a prospective active surveillance study at the Royal Marsden Hospital NHS Foundation Trust. The methods for selecting samples for biopsy TMA construction from patients with prostate cancer entered into active surveillance have been described previously.1 All patients gave their written consent to take part in the active surveillance study, which was approved by the local ethics committee.

The new procedure consists of two steps. In the first step, biopsy donor blocks were constructed using previously reported methods,² with only five biopsy checkers per block (figure 1A–F). The height of the ‘checker’ was specifically kept at around 4 mm (figure 1A, B) as previously reported.³ A variation in the new procedure was that the end of the core was painted with a red dot to identify the core end after embedding (figure 1C), and the side of the checker opposing the biopsy core was painted blue (figure 1D) for orientation. A finished biopsy ‘checker’ (figure 1E) and donor block (after paraffin wax embedding) (figure 1F) are shown.

In the second step, we use the conventional TMA construction techniques described by Kononen et al⁴ to punch out the needle biopsies from the donor block and reset them in a recipient wax block. A hollow needle punch was pushed into the donor block using an MTA1 Manual Tissue Arrayer (Beecher Instruments, Silver Spring, Maryland), to produce a 1.5 mm diameter core with 4 mm depth (figure 2A) that encompassed a biopsy specimen: the red marker dot (figure 1F) was used in each case to define the position that was punched. Each donor core was then transferred to a 1.5 mm diameter, 4 mm deep hole that had been punched into a recipient wax block (figure 2B). In total, a maximum of 104 cores (3×13) could be arrayed in each recipient block. Usually a number of blank spaces were left in each recipient block defining a unique pattern for block identification. This transfer procedure was usually highly efficient: a detailed examination of the many of the donor blocks following transfer failed to identify residual tissue. The only problems encountered were with very curved biopsies specimens that were not...
Figure 1  Construction of biopsy donor blocks. For clarity, the formalin-fixed prostate cancer needle biopsy has been coloured green. A malignant portion of the biopsy was marked and represented here by a black rectangle (A). The biopsy was then cut from its original block with a longitudinal length of about 4 mm to create a ‘checker’ (B). The cancerous end of the biopsy ‘checker’ was coloured red (C), and the opposing side to the biopsy was coloured blue (D) for orientation. A finished checker (E) and donor block after embedding (F) are pictured.

Figure 2  Biopsy tissue microarray (TMA) construction. An MTA1 manual tissue arrayer was used to punch out a biopsy core from a donor block (A). The biopsy core was then transferred to a recipient wax block (B). (C) Example of a finished biopsy TMA with (D) corresponding H&E. A black cross denotes the position of blank spaces.
suitable for arraying using this procedure. Preparation and sectioning of biopsy TMA blocks were carried out exactly as described previously. An example biopsy TMA block constructed by this method is shown in figure 2C, and the corresponding H&E section is shown in figure 2D. The method was initially used to construct biopsy TMAs in a 13×7 format (104 cores), but this created brittle block edges, and we consider the 91 (13×7) pattern to represent the most robust format. We have now used this procedure to construct 12 TMAs from around 1000 biopsies selected from Active Surveillance and MRC RT01 Radiotherapy trials including the coring and resetting of all biopsies in low-density TMAs constructed using our original method.

**DISCUSSION**

If biomarkers are to be used clinically for stratifying prostate cancer, they must be tested and validated in tissue obtained from the patient at the time of diagnosis, which usually only includes blood, urine and transultrasound-guided prostate needle biopsy samples. Examination of the prostate biopsy samples yields valuable information including Gleason grade and extent of disease, which facilitate decision-making on the appropriate treatment. The platform that we have developed additionally allows the use of formalin-fixed needle biopsies in multiplex analysis of biomarkers in the precise setting in which they would be used clinically. The multiplex analysis of biomarkers is critical because it is probable that a combination of biomarkers, rather than a single biomarker, will provide the best prognostic or diagnostic information. Needle biopsies are also taken at the time of diagnosis from many other human malignancies, including oral cancer, breast cancer and lymph-node metastases, so the techniques developed here for prostate cancer may also have relevance to other diseases.

**REFERENCES**