

REVIEW ARTICLE

Pathology in prostate research: Optimizing tissue quality

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Abstract

The collection of tissue from the prostate gland for research creates unique challenges in the identification of cancer and in preserving pathological material. *Value and uses of formalin fixed tissue.* Formalin fixed paraffin embedded (FFPE) tissue is often available in abundance after pathological processing and reporting of specimens but is limited in value for detailed molecular tests. Tissue micro-array if carefully performed is a helpful technique for examining many FFPE specimens with immunohistochemical or fluorescence *in situ* hybridization tests. *Value and uses of frozen tissue.* The collection of fresh tissue prior to formalin fixation and later validation samples of fresh prostate cancer is difficult as prostate cancer is very difficult to identify macroscopically on cut prostate specimens. Also, the act of manipulation and dissection of the gland while fresh and without compromising surgical margins is challenging. Methods which have been used to dissect the fresh prostate gland and also collect fresh tissue from other prostatic specimens are discussed. The ethical challenges of collecting research tissue without compromising patient care are discussed. *Conclusions.* Prostate cancer tissue banks, particularly of frozen tissue are still relatively few in number. Enhanced collection methods which do not prohibit full pathological examination are available but require expertise to maximize their potential.

Formalin fixed paraffin embedded tissue

Formalin fixed and paraffin embedded tissue (FFPE) allows a variety of molecular techniques to be assayed after histological diagnosis. Storage can be undertaken at room temperature and the vast majority of epitopes will remain stable even after 10–20 years or more in paraffin blocks. A number of factors may affect this. Firstly if another fixative such as Bouin's is used, this may cause loss or different expression of markers [1]. Secondly, after FFPE blocks have been cut onto slides there is a loss of strength of signal with some antigens. For instance, HER-2 is known to degrade within a few weeks of sections being cut [2]. It has been suggested that if unstained slides are stored then they are preserved from degradation by storing at a low temperature or an oxidation free environment [3].

Tissue microarrays (TMA) allow multiple specimens to be examined on a single slide while preserving their identity and also allowing a single immunohistochemical experiment to be performed on the same tissue [4,5]. It has proven a valuable

technique for high throughput of multiple tumors but nevertheless requires careful technical preparation. Areas for microarray need to be identified on adjacent Haematoxylin and Eosin sections, preferably by a pathologist. This is especially true for prostate carcinoma where diagnosis is often challenging, especially in low grade disease. Secondly the heterogeneity of tumor needs to be reflected in sampling. This will include sampling of the index tumor. As Gleason scoring has shown, prostate cancer shows unique heterogeneity. At least three areas of tumor should be sampled and all grades of tumor, if different grades are present [6]. TMA is a technique frequently used in TURP and radical prostatectomy specimens. However recently it has been shown to be possible to create TMAs from biopsy material as well as TURP and RP specimens [7].

Fresh tissue

Molecular profiling techniques such as real-time PCR and microarrays require the harvesting of high quality tissue, yet the process of formalin fixation

results in non-specific degradation of delicate intracellular molecules, particularly RNA [8,9]. Therefore although essential for cell culture studies, the collection of fresh tissue is also greatly preferable for DNA and especially RNA studies. However, the collection of fresh prostatic tissue, especially tumor tissue is far more challenging than tissue collection of most other tumors. A major challenge is to identify tumor at gross examination which is often very difficult or impossible in prostate cancer. Thus, morphological verification is absolutely mandatory. It must in no way compromise the diagnostic or prognostic information than can be extracted from the tissue. Therefore the ability to extract fresh tissue depends upon the surgical procedure undertaken.

TRUS biopsy

18 gauge needle biopsies are too small to identify cancer macroscopically, and inevitably the whole of the core is needed for histopathological processing. Due to the insensitivity of PSA screening and lack of precision of needle biopsies, only about 30–40% of TRUS biopsy procedures lead to the identification of cancer [10]. Methods of collecting fresh tissue from TRUS include the collection of extra needle cores for research. However this may pose ethical problems, especially if cancer is identified in the cores, while the specimens taken for routine FFPE processing are negative. It is likely to be useful only in extensive or high grade tumors. Some groups have attempted to use tissue imprints from biopsy cores [11] by transferring the superficial cells onto a surface. However, this also will tend to identify only high grade tumors.

TURP collection

As few pathology departments process all the tissue removed at TURP, there is usually scope to collect fresh tissue from these operations. However this is usually hyperplastic tissue from the transition zone or non-neoplastic central zone tissue. Although it might be considered an adequate ‘normal’ control tissue, it must be remembered that most of the tissue is not from the peripheral zone, where the majority of prostatic cancers arise. Techniques for sampling fresh tissue from TURP specimens include sampling the middle of a TURP chip while processing the outer portions routinely to correlate the morphology with the fresh tissue [12]. Occasionally prostatic cancers are operated upon using this method, especially in fast growing tumors which are not amenable to radical therapy, in cases of bladder outflow obstruction. In these cases, abundant tumor tissue is available. However, it should be remembered that these

tumors are unlikely to be representative of the full spectrum of prostate cancer. They are usually hormone pre-treated and occupy an unusual part of the prostate cancer spectrum, being tumors that have spread rapidly locally but have not yet resulted in death by metastatic spread.

Radical prostatectomy specimens

The harvesting of validated prostate tissue from radical prostatectomy specimens, probably represents the optimal method of the accrual of tissue from low risk prostate cancer. However the macroscopic dissection of the prostate gland is difficult and critical attention needs to be paid to margin status and capsular invasion. Therefore any dissection should always be conducted by a pathologist according to an agreed protocol.

A number of methods are currently in use to collect fresh tissue. Some centres use 18 gauge biopsies on the fresh gland before fixation in formalin [13]. This has the advantage of less distortion of the gland and minimal disruption of the surgical margin. However disadvantages are that little tissue is sampled and immediate correlation with macroscopic internal appearances of the gland are not possible. Furthermore, it is very difficult to localize the tumor as the majority of prostate cancers at radical prostatectomy are currently T1c tumors, i.e. non-palpable disease.

Another technique includes immediate sectioning of the gland with the dissection of suspicious areas of cancer from the center of the specimen where there is less danger of the disruption of surgical margins [14,15]. Disadvantages include the subsequent potential distortion of the specimen in formalin rendering assessment challenging, and the fact that increasingly prostate cancers are too small to identify macroscopically. A method has been described for harvesting tissue from cut surfaces without compromising surgical margins [16].

Radical cystectomy specimens removed for urothelial carcinomas are also a potential source of prostate tissue. Although small prostate cancers may be found in many cases, they represent a potential source of ‘normal’ controls and do not require such rigorous assessment of margin status of the prostate.

Storage of fresh prostate tissue for research

Due to rapid autolysis, tissue should be stabilized as soon as possible after removal from the patient. Common methods include snap freezing of tissue and storage at -70°C . Cooling the specimen immediately in theater by placing it on ice may be helpful. However for transport this is often cumbersome and the

development of an RNA stabilization solution RNAlater may be used if RNA is to be analysed [15]. A disadvantage of RNAlater is that it impairs the interpretation of morphology. Correlation of all tissue with either frozen sections or adjacent FFPE tissue is important if cancer is to be properly validated [17].

When harvesting for cell culture, morphological verification with frozen section or RNAlater fixed material is not possible as the harvested tissue needs to remain fresh. This may also be the case with tissue intended for proteomic analysis. A morphological validation can then be carried out using cytological sampling technique [18].

Ethical issues

The ethical issues involved in the collection and use of fresh tissue will vary greatly between countries [19–21]. However they are underpinned by an ethos that no patient should suffer harm from the research, and that their confidentiality must be ensured. Ethical approval in the country of origin should always be obtained, and depending on the procedure and country, consent by the patient is required. Harm is conceivable if the patient undergoes unnecessary procedures: for instance research orientated TRUS biopsies with no diagnostic use or intent. The patient may undergo indirect harm if research manipulation of tissue renders a specimen difficult to interpret for margin status.

Conclusions

Harvesting the prostate, especially cancer tissue, for research creates problems not encountered in other organs and cancers, mostly secondary to the unique methods by which prostate cancer is diagnosed and sampled. However by using the variety of techniques suggested above and with cooperation of local pathologists it is possible to collect both frozen and FFPE tissue banks which can be used for future research programs.

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