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Technical report

Microdissection of stained archival tissue

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Abstract

In many tissues the preinvasive stage of neoplastic progression can be identified histologically as dysplasia or in situ disease. There is much interest in defining the molecular events associated with the early stages of neoplasia. Retrieval of histologically recognisable preinvasive neoplastic tissue uncontaminated by inflammatory or stromal cells is important for genetic studies using polymerase chain reaction (PCR) assay. A novel method for microdissection is described in which 10 μm sections are dewaxed, stained with haematoxylin and eosin, dried, covered with Sellotape, and the tissue cut out using a scalpel blade under direct visual control. The method is quick, eliminates problems of operator tremor, preserves the architecture of the microdissected tissue (for photographic documentation) and requires no special equipment. The presence of Sellotape and adhesive in the reaction mixture has no detrimental effect on the ability to extract DNA or to perform PCR.

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Although it is known that fixation in formalin and processing of tissue to paraffin wax degrades DNA, it is possible to extract DNA sequences of up to 200 base pairs from wax embedded archival material.[1] The sequences of DNA extracted can then be amplified using polymerase chain reaction (PCR) to provide specific information about allele loss and point mutations. There are major advantages to performing molecular analysis on material retrieved under histological control. The nature and purity of the material can be monitored (by photography if necessary). For example, when pieces of tumour are taken from a grossly visible mass it is difficult to determine how much of the sample is tumour and how much is stroma. The purity of the tissue can only be checked retrospectively if a histological section is taken before it is digested. For studies of loss of heterozygosity it is important that tumour tissue only is analysed as contamination with germline background stromal or inflammatory cells may mask the allelic loss in the gels.[2] The requirement for histological confirmation and difficulties with contamination have led investigators to retrieve pure tumour tissue from glass slides using microdissection techniques.[3] This involves identifying the tumour required, cutting around it and removing it to an eppendorf tube for PCR. A number of methods to achieve this have been developed.[3 4] We report a novel method for retrieving histologically defined pieces of archival tissue for PCR analysis. Although this method could be used for any tissue type we illustrate the technique by sampling individual breast ducts containing ductal carcinoma in situ (DCIS) of the breast for PCR analysis.

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Materials and methods

MICRODISSECTION TECHNIQUE

A suitable archival tissue block containing the required tumour is selected (in this case containing ducts with DCIS). A 5 μm standard section is cut, dewaxed, and stained with haematoxylin and eosin. The next section is cut at 10 μm onto a standard (non-coated) glass slide, dewaxed, and stained with haematoxylin and eosin. The section is allowed to dry for 10 minutes but is not mounted and coverslipped. The area of the section is then covered with Sellotape, which is pressed firmly onto the tissue section with a finger. The slide can then be viewed under a dissecting microscope and the area (in this case a duct) for microdissection can be easily identified (fig 1A). The optical quality is less than that obtained through a glass coverslip and mounting medium but is acceptable for easy identification of the target tissue.

The required area is simply cut out using a Swann-Morton number 11 blade under direct visual control through a dissecting microscope. One of the problems with microdissection is operator tremor but with this technique once the blade is embedded in the Sellotape it can be pressed hard onto the underlying glass, which eliminates tremor. The blade is then drawn through the Sellotape around the duct being dissected. The blade is held in the dominant hand, the slide being held and steadied in the other hand. The slide can be rotated to allow cutting around the curves of the duct. A precise

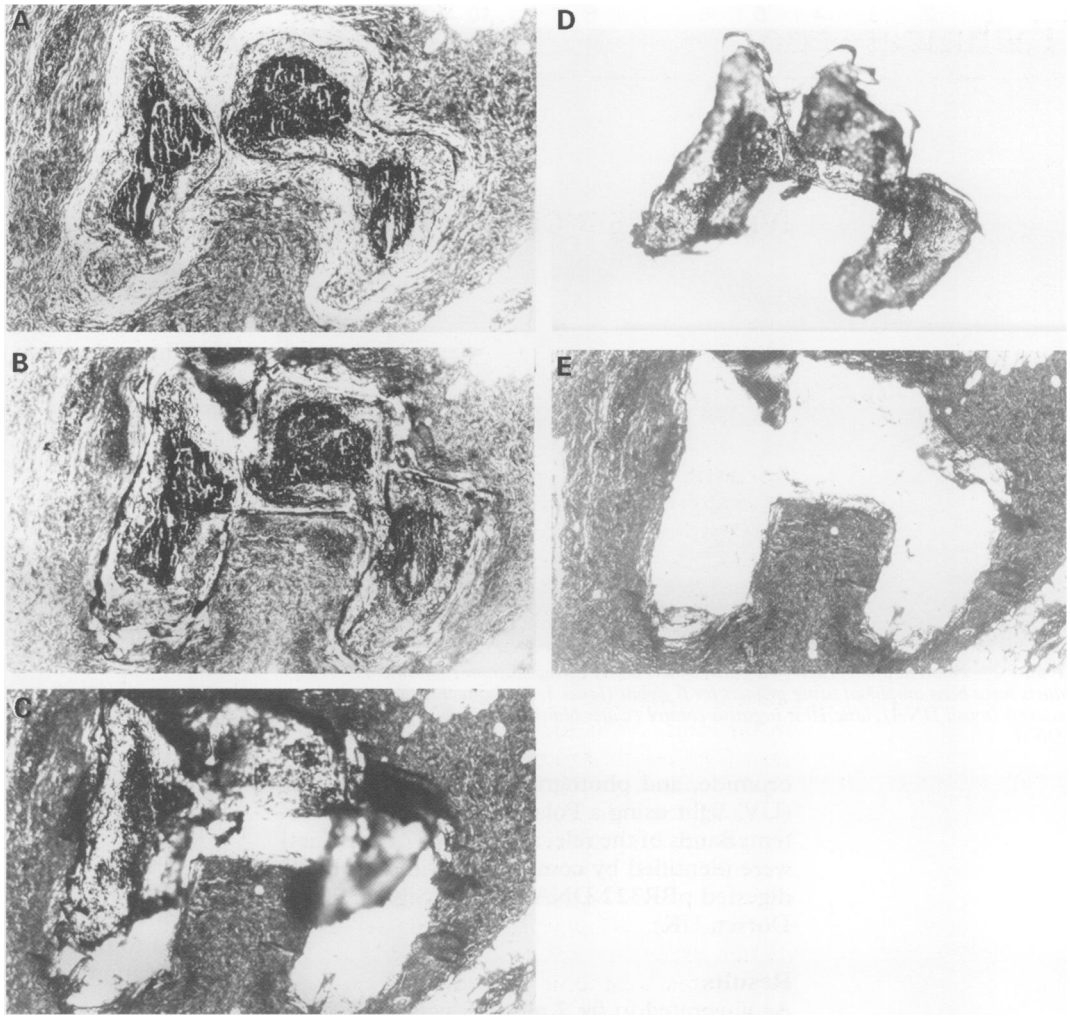

Figure 1 (A) Distended breast ducts containing DCIS with areas of central necrosis. The tissue has been stained with haematoxylin and eosin and covered with Sellotape. Morphology is adequate for dissection. (B) The ducts have been cut around the periphery (basement membrane) through the Sellotape. (C) The microdissected area is mobilised from the surrounding tissue. (D) Microdissected piece of Sellotape with adherent tumour photographed for documentation. (E) Hole remaining in the section after microdissection is complete.

cut around the duct can be made through the Sellotape (fig 1B).

The cells within the duct become adherent to the thick viscid adhesive on the underside of the Sellotape. In many cases the tissue will simply lift off the underlying glass slide. If the cells are adherent it is possible to scrape them off the glass gently with the tip of the blade and push them into contact with the thick viscid adhesive on the underside of the tape. The process is akin to using a soft putty rubber as an eraser (fig 1C). When the duct content is embedded within the adhesive the whole piece of dissected Sellotape can be picked up with fine forceps and transferred to an eppendorf tube. Frequently the morphology of the dissected tissue is retained beneath the Sellotape and can be photographed before it is placed in the eppendorf tube for documentation (figs 1D and E).

DNA EXTRACTION

The microdissected material being already dewaxed does not require dewaxing. Microdissected tissue adherent to Sellotape fragments was incubated for 72 hours at 37°C in a digestion buffer containing 50 mM KCl, 10 mM Tris HCl (pH 7.5), 1 mM EDTA, 1% (wt/vol) sodium dodecyl sulphate, and 200 μg/ml proteinase K. The mixture was then heated to 95°C for 10 minutes to inactivate the proteinase K and aliquots were used directly for the PCR.

MARKERS AND PCR TECHNIQUE

The reaction mixture consisted of primers KM38 and PCO_3 for β globin[5] each used at 1 μM, 2 μl of DNA extract, 200 μM of each deoxynucleotide triphosphate (dATP, dCTP, dGTP, dTTP), 50 mM KCl, 10 mM Tris HCl (pH 9.0), 1.5 mM $MgCl_2$ (optimised for maximum yield and specificity), 100 μg/ml bovine serum albumin (BSA), and 0.5 U of Taq polymerase (Perkin Elmer, Beaconsfield, UK) made up to a total volume of 50 μl with sterile distilled water and sealed with 1–2 drops of mineral oil. Forty PCR cycles were performed on a Perkin Elmer 4800 thermal cycler, each cycle consisting of a denaturing step at 93°C for one minute, an annealing step at 56°C for one minute (optimised for maximum yield and specificity), and an elongation step at 73°C for one minute. Following the 40 cycles there was a five minute period at 73°C to ensure complete extension and annealing of the PCR products. Aliquots (15 μl) were then analysed by electrophoresis in a 10% polyacrylamide gel at 200 V for one hour, stained with ethidium

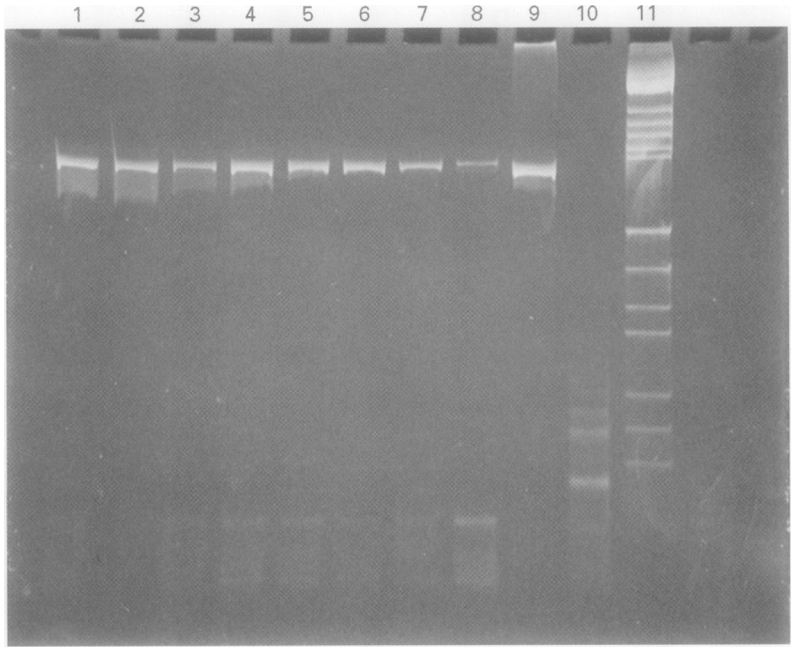

Figure 2 Polaroid photograph of a gel in which DNA extracted from eight microdissected ducts have been amplified using primers for β globin (lanes 1–8). Lane 9 is the positive control (tonsil DNA), lane 10 is negative control (water blank), and lane 11 is marker DNA.

bromide, and photographed under ultraviolet (UV) light using a Polaroid DS34 camera system. Bands of the relevant size (167 base pairs) were identified by comparison with an HaeIII digested pBR322 DNA marker (Sigma, Poole, Dorset, UK).

Results

As illustrated in fig 2 all the ducts show good amplification with intensely staining bands of the expected size (167 base pairs).

Discussion

Microdissection of tissue from archival histological material is a powerful technique for the investigation of the genetic events involved in carcinogenesis.[2 5] Microdissection is particularly powerful because pure tissue of known composition can be obtained from the earliest histologically recognisable stages of the preinvasive neoplastic process. Some of these preneoplastic proliferations and in situ neoplasms occur in epithelia above the basement membrane, the volume of tissue may be very small, and it is extremely difficult to obtain histologically confirmed dysplastic cells in any other way. In the technique described here the dissection is performed directly on a haematoxylin and eosin stained section and the excised tissue can be subsequently photographed for documentation (fig 1D). No dewaxing of the tissue in the eppendorf is required, the tissue remaining on the slide is protected by Sellotape and can be filed allowing further microdissections from the same slide when needed.

In illustrating this technique we have used DCIS of the breast as an example but the technique is applicable to any tissue type. It is likely that microdissection of in situ malignancy at other sites will require cutting of straight lines (along the basement membrane) that should be easier than the circular cuts required to excise DCIS. We have used primers for amplification of the β globin gene to demonstrate that the DNA extracted from the microdissected tissue was of sufficient quantity and quality to allow amplification by PCR. This cannot guarantee that amplification using other primer sets will necessarily be successful and of course amplification conditions for each primer set need to be optimised individually.

We believe that the technique described represents an advance because we have found that it is quick to perform and eliminates tremor problems. The method is cheap requiring minimal equipment. In addition, slides can be returned to the archive and so it is well suited to application in routine surgical pathology laboratories.

1 Bianchi AB, Navonne NM, Conti CJ. Detection of loss of heterozygosity in formalin-fixed paraffin-embedded tumor specimens by the polymerase chain reaction. *Am J Pathol* 1991;**138**:279–84.
2 Stratton MR, Collins N, Lakhani SR, Sloane JP. Loss of heterozygosity in ductal carcinoma in situ of the breast. *J Pathol* 1995;**175**:195–201.
3 Zhuang Z, Bertheau P, Emmert-Buck MR, Liotta LA, Gnarra J, Lineham WM, *et al.* A microdissection technique for archival DNA analysis of specific cell populations in lesions < 1 mm in size. *Am J Pathol* 1995;**146**:620–5.
4 Martin JE, Swash M, Mather K, Garofalo O, Dale GE, Leigh PN, Anderton BH. Microdissection: a novel method for the study of intracellular inclusion bodies. *J Pathol* 1990;**160**:77–9.
5 Slack DN, McCarthy KP, Wiedemann LM, Sloane JP. Evaluation of sensitivity, specificity and reproducibility of an optimised method for detecting clonal rearrangements of immunoglobulin and T-cell receptor genes in formalin-fixed paraffin-embedded sections. *Diagn Mol Pathol* 1993; **2**:223–32.