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Short reports

Pulsed field gel electrophoresis on frozen tumour tissue sections

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Abstract

The application of pulsed field gel electrophoresis (PFGE) to the molecular genetic analysis of solid tumours has been restricted by the requirement for whole single cells as a DNA source. A simple technique which allows for the direct analysis of histologically characterised solid tumour material by pulsed field gel electrophoresis was developed. Single frozen tissue sections obtained from colonic carcinomia specimens were embedded without further manipulation in molten, low melting temperature agarose. The tumour DNA contained within the agarose plug was subjected to restriction enzyme digestion and PFGE. Sufficient high molecular weight DNA is yielded by this method to obtain a hybridisation signal with a single copy probe. Histological examination of adjacent tissue sections may also be carried out, permitting correlation between molecular analysis and tumour histology.

Pulsed field gel electrophoresis (PFGE) is a powerful technique for the molecular characterisation of chromosomal deletions and translocations that occur in human neoplasia.[1] There are, however, several technical problems that need to be resolved in order for this technique to be widely applicable to the study of chromosomal rearrangements in solid tumours.[2] One fundamental difference between conventional gel electrophoresis and PFGE is that DNA obtained from solid tissue by standard phenol extraction is not suitable for molecular analysis by PFGE because of the possibility of DNA shearing inherent in this method. Single cell suspensions are, therefore, an absolute requirement for all PFGE studies. This problem has been addressed, in part, by the development of methods that involve either the isolation of whole nuclei[3] or the generation of single cell suspensions[4] from solid tissue. Both techniques are laborious and time consuming. Alternatively, cell lines derived from solid tumours can, where available, be used for PFGE studies.[5] The use of tumour derived cell lines is not ideal, however, as methylation differences arising during cell culture can be a misleading source of altered PFGE fragments.[6]

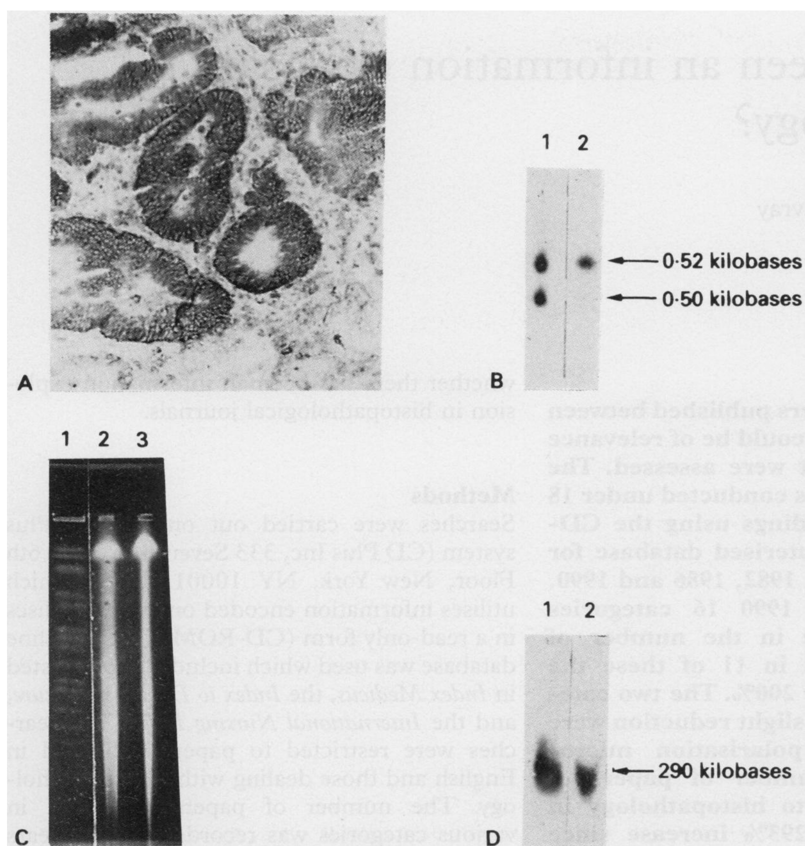
We investigated the use of tissue sections, obtained from frozen biopsy specimens of cancer of the colon and embedded without further manipulation in agarose, as a DNA source for PFGE.

Methods

Specimens of colonic carcinoma, including adjacent normal mucosa, were collected and frozen in liquid nitrogen. Two single frozen sections (10 μm thick, 0·5 × 1 cm^2) were cut from each of the tumour and normal mucosa specimens, respectively. The first of each mirror section was retained for standard histological staining while the other, handled using chilled fine forceps, was embedded whole and without further manipulation in 20 μl of 1% molten, low melting temperature agarose (Seaplaque) in PBS-1 mmol EDTA in a 100 μl rectangular insert mould. Solidified inserts were placed into lysis solution consisting of NDS (10 mmol TRIS-HCl, 0·5 mol EDTA, and 1% Sarcosyl, pH 9·5), and proteinase K 1 mg/ml. Blocks were incubated for 24 hours at 50°C and this was repeated with fresh lysis solution for a further 24 hours. Blocks were then rinsed several times in NDS and stored at 4°C until use. Enzyme digestion, conventional electrophoresis, PFGE, Southern blotting, and hybridisation were performed according to standard methods.[7] The DNA present in the agarose block was digested with the rare cutter restriction enzyme *Ecl* X I for PFGE analysis and then separated in a 1·0% agarose gel on a CHEF II PFGE system (Biorad). The resulting filter was hybridised with a 5′ genomic probe for CSF-IR which is known to give a single band of 290 kilobases following DNA digestion with *Ecl* X I. For conventional electrophoresis, the DNA present in the agarose block was digested with the restriction enzyme *Taq* I and then separated in a 1·2% agarose gel. The resulting filter was hybridised with pYNZ22·1 which detects a variable number tandem repeat (VNTR) at 17p13·3, a locus (D1755) frequently deleted in cancer of the colon.[8]

Results

High molecular weight DNA was obtained from the single frozen tissue sections embedded in agarose. Observation of the ethidium stained gels following both PFGE and conventional electrophoresis showed complete DNA digestion with the enzymes *Ecl* X I (fig 1C) and *Taq* I, respectively. The resulting PFGE filter was hybridised with a 5′ genomic probe for



(A) Frozen section of cancer of the colon stained with haematoxylin and eosin. (B) Southern blot hybridised with the probe pYNZ22·1. Track 1: normal mucosal DNA digested with Taq I. Track 2: colon carcinoma DNA digested with Taq I (mirror section to A). (C) Ethidium-stained PFGE gel. Track 1: yeast marker Saccharomyces cervisiae. Track 2: peripheral blood DNA digested with Ecl X I. Track 3: colon carcinoma DNA digested with Ecl X I. (D) Resulting autoradiograph of same gel after hybridisation with 5' genomic probe for CSF-1R.

CSF-1R and a single band of the expected size of 290 kilobases was obtained (fig 1D). The filter obtained following conventional electrophoresis was hybridised to the probe pYNZ22·1. In an individual heterozygous for the *Taq* I RFLP detected by pYNZ22·1 two alleles were found to be present in the normal mucosa while the loss of the larger allelic fragment was clearly visible in the tumour specimen taken from the same individual (fig 1B). The mirror section partnering this tumour section is shown in fig 1A.

Discussion

We have investigated the use of single tissue sections taken from frozen biopsy specimens and embedded without further manipulation in agarose as a direct source of DNA for PFGE studies of solid tumours. We have shown for the first time that individual frozen sections obtained from colonic carcinoma specimens yield sufficient high molecular weight DNA to obtain a hybridisation signal on a PFGE filter using a single copy probe. The taking of mirror sections allows for molecular analysis and histological examination of the same area of the tumour. This method makes PFGE directly applicable to the study of genetic rearrangements and deletions occurring in solid tumours. Retrospective studies on stored frozen biopsy specimens represent one possible application for this method. We have also shown the suitability of this method for molecular studies using conventional gel electrophoresis. Although the extraction of DNA from small sections of frozen biopsy material by standard phenol extraction is well established,[9] this method has the inherent disadvantage of causing substantial loss of DNA. The use of frozen sections embedded directly in agarose obviates the need for further manipulation before enzyme digestion and results in no loss of DNA. This method may therefore be of considerable benefit in situations where only tiny biopsy specimens are available for study.

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