Application of microsatellite PCR techniques in the identification of mixed up tissue specimens in surgical pathology

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Abstract

A fragment of tumour was erroneously mixed up with an endometrial biopsy. Microsatellite polymerase chain reaction (PCR) clearly demonstrated the extraneous nature of the fragment. Microsatellite PCR may be useful for the identification of mislabelled or mismatched tissue fragments in surgical pathology specimens.
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Keywords: mislabelled tissue; mixed up specimens; microsatellite PCR

The presence of extraneous (foreign or contaminant) tissue in microscopic sections may cause major problems in the pathological diagnosis.[1–6] Rarely, surgical pathology tissue specimens may get mixed up during the process of gross description, embedding in paraffin, sectioning tissue blocks, mounting tissue sections on slides, or even staining in the same jar.[1] In some other cases, however, misidentification of a surgical specimen may occur during registration or because of erroneous labelling. Although such errors ought to be avoided by good laboratory practice, mistakes do occur. A recent study undertaken by the College of American Pathologists estimated that extraneous tissue occurs in 0.6–2.9% of surgical pathology specimens.[1] In some cases an erroneous diagnosis of cancer, based on the presence of contaminant mixed up tissue, may lead to unnecessary diagnostic explorations or to treatments.[5]

Several immunohistochemical and molecular methods have been reported as being useful in the identification of mismatched or mislabelled tissue specimens.[2–6] In this paper, we describe the value of microsatellite polymerase chain reaction (PCR) in the identification of a contaminant tumour fragment in an endometrial biopsy obtained from a postmenopausal woman. In this case, the unusual morphological appearance of the fragment led to the suspicion of a foreign or contaminant tissue.

Case report

A 64 year old woman underwent an endometrial biopsy after an episode of vaginal bleeding. Microscopic examination of the biopsy revealed multiple small fragments of superficial or atrophic endometrium together with a tissue fragment (2 mm in largest diameter) that was initially thought to correspond to a tumour (fig 1). The fragment was composed of a monotonous and dense population of medium sized cells arranged in vague fascicular and pavimentous patterns. Although mitotic figures were rarely seen, the cells showed occasional pleomorphism.

The pavimentous appearance of the biopsy led to the consideration of a large differential diagnosis that included rare variants of endometrial tumours and metaplasias. However, intensive microscopic screening of the biopsy revealed several morphological features that led to the suspicion of a mixed up specimen: (1) in some areas the cells were distributed in a whorled arrangement; (2) tumour cells showed nuclear inclusions similar to those of arachnoidal cells; and (3) there was a widespread presence of eosinophilic amorphous material resembling that characteristically found in the so called secretory meningioma.

Once there was suspicion that a fragment of a meningioma had been erroneously mixed up with the endometrial biopsy, a search in the surgical pathology files was undertaken to identify a tumour that was the possible source of contamination. Unfortunately, on the same day as the endometrial biopsy no meningiomas had been received in the laboratory, nor had

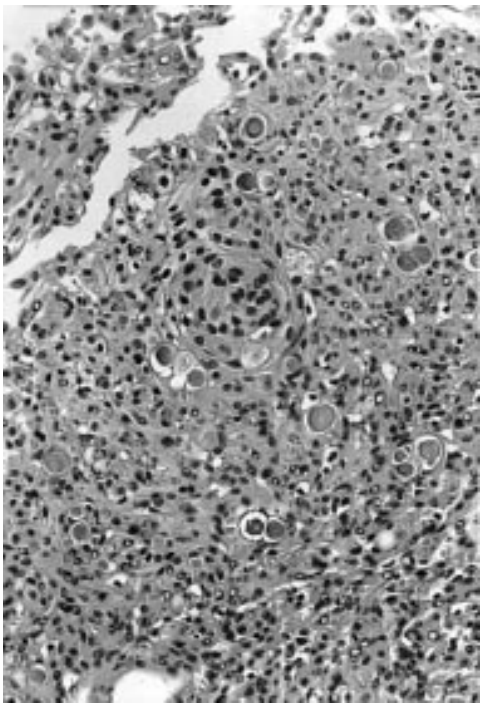

Figure 1 High power view of the presumed contaminant tissue fragment. The morphological features resemble those of a secretory meningioma.

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they on the day before or on the day after. The most recent meningioma on record had been received two days earlier. Microscopic examination of that tumour showed pathological features identical to those in the questionable fragment of endometrial biopsy. However, comparison of the laboratory processing of the two biopsies showed that the various stages (gross inspection, embedding, sectioning, and staining) had been done at different times. Furthermore, the two biopsies were managed by different histotechnologists or pathologists during the entire process. These discrepancies led us to perform molecular analysis to confirm the mixed up nature of the fragment.

Microsatellite PCR

To make selection of the microsatellites to be analysed easier, we started by comparing the different microsatellite profiles in DNA obtained from peripheral blood from the two patients. Only after selecting a small number of informative microsatellites was DNA obtained from the paraffin block by microdissection subjected to analysis.

For the microsatellite PCR, genomic DNA was isolated from peripheral blood and paraffin embedded material by standard methods.[9] DNA was analysed at seven different $(CA)_n$ repeats (Research Genetics Inc) localised on chromosome 3 (D3S1283; D3S1286; D3S1314), 13 (D13S171; D13S263; D13S267), and 18 (D18S58). Each PCR was performed in a 25 µl reaction mixture containing 100 ng of DNA, 166 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl (pH 8.8 25°C), 0.1% Tween-20, 1.5 mM $MgCl_2$, 10 pmol each primer, 0.2 mM each dNTP, and 1.5 units of Taq DNA polymerase (Amplitaq Gold, Perkin Elmer). The PCR reactions were carried out in a OmniGene Hybaid Thermocycler for 35 cycles of amplification (94°C for 30 seconds, 50–52°C for 30 seconds depending on the primer, 72°C for one minute; first cycle 95°C for 10 minutes; last cycle 72°C for 10 minutes). Following PCR, 10 µl of each PCR product were then mixed with 15 µl of loading buffer containing 95% formamide, 10 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol and heat denatured at 95°C for 10 minutes. Then 6 µl of the denatured samples were loaded onto an 8% denaturing polyacrylamide gel containing 8.3 M urea, and run at 1800 V for 4.5 hours. PCR products were capillary blotted onto Hybond N^+ membrane (Amersham) and DNA was fixed by a simple alkali treatment. The membranes were analysed using the ECL gene detection system (ECL Amersham), according to the specifications of the Amersham kit with minor modifications. The detection system was enhanced by chemiluminiscence with a 5'-end labelling method, and the filters were detected on blue light sensitive films.

Results

The best results were obtained with microsatellites D3S1286, D18S58, and D13S171 (fig 2). The pattern of bands of the presumed contaminant fragment matched the DNA extracted from the peripheral blood of the patient who had the meningioma and also the DNA obtained from the meningioma itself. In contrast, the pattern of bands corresponding to the peripheral blood of the patient who under-

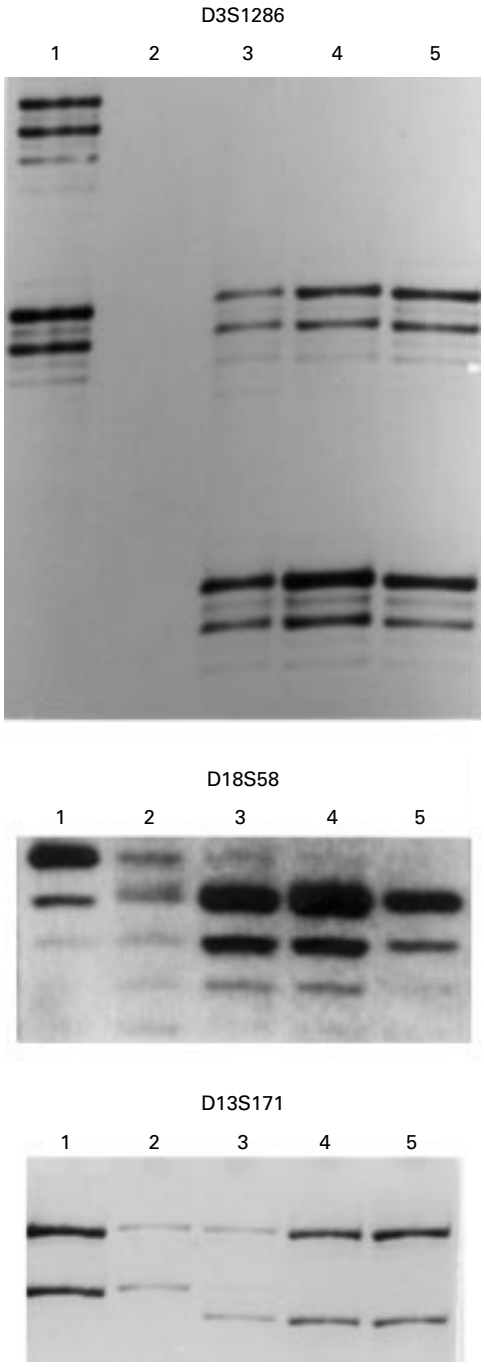

Figure 2 Representative picture of the gels corresponding to microsatellites D3S1286, D18S58, and D13SS171. Lanes 1 were DNA obtained from peripheral blood of the patient that presented with vaginal bleeding. Lanes 2 represented DNA extracted from the atrophic endometrium of the same patient as lanes 1. Lanes 3 corresponded to DNA obtained from the presumed contaminant tissue fragment. Lanes 4 represented DNA isolated from the meningioma that was interpreted as the possible source of contamination. Lanes 5 were DNA from peripheral blood of the patient with the meningioma. No amplification was obtained from DNA of the atrophic endometrium (lane 3) for marker D3S1286, because of the scarcity of the material obtained in the biopsy. The results clearly show that the suspicious fragment (lanes 3) did not match the DNA obtained from peripheral blood (lanes 1) and endometrial tissue (lanes 2) of the patient who underwent the endometrial biopsy.

went the endometrial biopsy matched that obtained from the atrophic endometrium for markers D13S171 and D18S58. No amplification was obtained from DNA of the atrophic endometrium for marker D3S1286 because of the scarcity of the material obtained in the biopsy. Results from the other four markers yielded similar results; however, in some of them the typical background laddering of microsatellite PCR from DNA obtained from paraffin embedded material made the interpretation difficult. Nevertheless, the results clearly showed that the putative contaminant did not match the DNA obtained from the patient who underwent the endometrial biopsy.

The possibility that the mixed up specimen was not from the patients whose blood samples were compared, but from another (third) patient is very small. At present, it is impossible to calculate such a probability, because of the lack of studies on the distribution of the allele frequencies for each locus in our population. However, the polymorphic rate of these markers (D3S1283, 0.71; D3S1286, 0.89; D3S1314, 0.87; D13S171, 0.73; D13S263, 0.84; D13S267, 0.69; D18S58, 0.74) indicates that such a probability is very small.

Discussion

Several immunohistochemical and molecular methods have been proposed to identify mislabelled or mismatched tissue specimens. These include immunostaining of ABO blood determinants[2] and HLA class I antigens, and PCR for human leucocyte antigen (HLA) DQ α and low density lipoprotein receptor genes.[3] Even a commercially available kit based on the polymerase chain reaction is now available.[4 5] Furthermore, the value of polymorphic microsatellite markers in the identification of mixed up bladder biopsy specimens was also recently postulated.[6] In this paper we have attempted to provide more evidence that microsatellite PCR is a useful tool for identifying such foreign contaminant tissues in surgical pathology specimens. Microsatellite PCR can be used for this purpose in all laboratories where the technique has been implemented appropriately.

Microsatellites belong to the family of repetitive non-coding DNA sequences, together with satellites and minisatellites.[8] Microsatellites are tandem arrays of short stretches of nucleotide sequences (two to six base pairs), usually repeated between 15 and 30 times.[8] They are highly polymorphic, and allele sizes in populations characteristically show multiple size classes distributed around the population, with Mendelian codominant inheritance.[9] The polymorphic rate of dinucleotide microsatellites increases with the increase of the average number of repeats. The human genome is thought to contain approximately 12 000 highly polymorphic microsatellites.

Microsatellite PCR follows the general principles of genomic DNA amplification.[8] A common problem with dinucleotide repeats is the presence of additional (stutter) bands beside the microsatellite band, differing by one or two base pairs, which may pose problems in interpretation of gels[8]; this is particularly important in DNA obtained from paraffin embedded tissues. To avoid the presence of spurious bands owing to mispriming, it is very important to use annealing temperatures that are as high as possible and to reduce extension times to the minimum.

Because microsatellite alleles segregate in a Mendelian fashion in families, microsatellites are regarded as excellent candidates for personal identification systems in medical and forensic applications.[10] In the present article we have tried to show that these techniques could be used in the identification of extraneous tissue in surgical pathology specimens. The application of this method with a small number of markers may be enough to show that the putative cross contaminant is extraneous tissue; this is particularly important in small fragments like the example presented here (it measured 2 mm in its largest diameter). The precise identification of the specimen that was the source of the contamination may require the use of additional markers, depending on the degree of polymorphism of these markers in the general population.

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