SHORT COMMUNICATION

Bladder cancer cell line cross-contamination: Identification using a locus-specific minisatellite probe

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Cross-contamination of cells in culture is a common occurrence. Because mammalian cells in monolayer culture can be difficult to distinguish morphologically, cross-contamination can pass undetected. The cross-contaminating cell line rapidly takes over, and within two or three passages the original cells are undetectable. Consequently, many studies have been published using a cell type different from that thought to have been studied. A classic example of this occurred during the early reports of neoplastic transformation of mouse cells by DNA from human cancer cells. Transfection of DNA from putatively the same human bladder cancer cell lines did not always result in neoplastic transformation of mouse 3T3 cells (Peruch et al., 1981; Der et al., 1982; Goldfarb et al., 1982; Parada et al., 1982). We then demonstrated by isozyme and HLA typing that some of these lines were cross-contaminated (O’Toole et al., 1983) and only those contaminated by the human bladder cancer cell line T24 produced high levels of neoplastic transformation following transfection.

The isozyme typing seemed to provide a satisfactory explanation for the disparities between the transfection studies, but this was not the end of the story. It was reported subsequently that some of these cross-contaminated sublines had a Y chromosome (Hastings & Franks, 1983; Lin et al., 1985), yet the T24 cell line was derived from a female. This anomaly was compounded by heterogeneity amongst the sublines in respect of their transplantability to nude mice (Hastings & Franks, 1981; Masters et al., 1986), tumour morphology after xenotransplantation (Masters et al., 1986), staining patterns with monoclonal antibodies (Bubenik et al., 1985; Trejdosiewicz et al., 1985), invasiveness in vitro (Kiefel, 1984) and cell size (Christensen et al., 1984). In addition, the isozyme data were flawed by a disparity at one locus between T24 and some of the cross-contaminated sublines, comparing earlier data (Povey et al., 1976) with that reported in 1983 (O’Toole et al.).

Recently a more powerful tool for discriminating between individuals and cell lines in culture has become available. DNA fingerprinting uses hybridization probes to detect length polymorphism of repeat DNA sequences, giving a pattern unique for each individual (Gill et al., 1985; Jeffreys et al., 1985a,b). We have applied a variation of this new technique, utilising a locus-specific minisatellite probe to determine conclusively whether the human bladder cancer cell lines that we studied by isozyme typing (O’Toole et al., 1983) were cross-contaminated by the T24 cell line. In addition, a Y-chromosome specific probe was used to investigate the enigma of the karyotypic studies.

Nine continuous cell lines derived from human tumours were included in this study, including T24 and the five putatively cross-contaminated sublines MGH-U1, MGH-U2, J82, HU456 and HU961T. In addition, two other bladder cancer cell lines, J82CO'T and RT112, and one testicular germ cell tumour cell line, SuSa, were tested.

DNA was obtained from ~100 million cells using a phenol extraction technique described by Pera et al. (1981). Contaminating RNA was removed by the sequential use of t1 and t2 RNases (Panasci et al., 1977). The purity of the DNA was estimated by determining the ratio of optical densities at 260 and 280 nm (Maniatis et al., 1982).

The plasmid pZ3, containing a locus-specific minisatellite sequence (Wong et al., 1986) was provided by Dr Alec Jeffreys. The 7.1 kb insert was released from the plasmid by digestion with Sau3A, and a probe was prepared by further digestion of this insert with Aul, generating a fragment of ~6.5 kb. This fragment contained virtually only the region of tandem repeats, lacking the Alu sequences and most of the single copy sequences present in the original insert (see map in Wong et al., 1986). Consequently it was not necessary to use human competitor DNA in the hybridization mix. The probe was purified from low melting point agarose and 50 ng labelled with [32P]dCTP (Amersham International) using random priming (Feinberg & Vogelstein, 1984). All restriction enzymes were obtained from Anglian Biotechnology.

DNA (5 μg) from each cell line was digested with Hind1 and electrophoresed for 24 h at 1.3 vcm⁻¹ in 0.6% agarose. The buffer used was Tris/Acetate/EDTA, pH 8.0 (Maniatis et al., 1982). The gel was blotted onto Gene Screen Plus (New England Nuclear) and hybridized to the labelled probe at 65°C without formamide as described in the manufacturer’s instructions. The filters were washed first in 2× SSC (0.15 M sodium chloride and 0.015 M sodium citrate), 0.1% SDS at 65°C, and then at 0.1× SSC, 0.1% SDS at the same temperature, before exposure to Kodak X-OMAT AR film overnight at ~70°C in the presence of an intensifying screen.

For the detection of Y-specific sequences the methods used were similar except that the DNA was digested with EcoRI and electrophoresed for 16 h in 1% agarose in buffer previously described. The probe used, CY84 (Wolf et al., 1986) was supplied by Dr Jonathan Wolfe. This probe has been shown to detect a 5.5 kb band which is specific for the Y chromosome (Wolfe et al., 1986).

The results obtained using the locus-specific hypervariable probe are shown in Figure 1. T24 and the five putative sublines all have an identical heterozygous pattern. RT112, J82CO'T and SuSa are distinct. The results using the probe CY84 are shown in Figure 2. The Y-specific 5 kb band is seen clearly in J82CO'T and SuSa, both derived from men, and is not seen in RT112 or T24, both of which were derived from women. The Y-specific band was not seen in any of the five putative sublines of T24, all of which were thought to have been derived from men.

These results should dispel any doubts concerning the identity of T24 and the five cell lines contaminated by T24. All are identical.

The two qualities needed in a test for cell identification are clear discrimination between individuals and stability of the
A feature in culture. Use of the probe \( p_{\mu 3} \) allows both these criteria to be satisfied. Using this plasmid Wong et al. (1986) examined 77 unrelated individuals and found at least 79 different alleles. Their data were consistent with the suggestion that there is one relatively common short allele, \( \sim 1 \text{ kb} \) long and with a gene frequency of \( \sim 0.16 \), and 103 other alleles all of which are equally rare. This would mean that the chance of two unrelated individuals having identical phenotypes when tested with this probe would be 1 in 1666. However, most identical individuals found would be homozygous for the common short allele. The chance of identity between random individuals if the short allele is not present (as in the case of T24) falls to \( \sim 1 \) in 5000. This is not as powerful as a discriminant as one of the polycore sequence 'fingerprinting' probes, where the chance of identity is about \( 3 \times 10^{-11} \) (Jeffreys et al., 1985a). It is also not as powerful as six independent locus-specific probes, used either sequentially or in a mixture, which give probabilities of identity between unrelated individuals of \( 10^{-16} \) and \( 6 \times 10^{-7} \) respectively (Wong et al., 1987). However, for practical purposes \( p_{\mu 3} \) is adequate and its application technically simple.

The data on the stability in cell culture of the loci detected by hypervariable probes are not yet as extensive as in the literature on isozymes. It has been suggested that these loci may be more mutable than others (Jeffreys et al., 1985b). However identical polycore 'fingerprints' were obtained from lymphoblastoid lines in culture and from direct DNA preparation from blood (Jeffreys et al., 1985b). Similarly, the loci are generally stable in the germline and in monozygotic twins (Jeffreys et al., 1985a). Tumour and normal tissue DNA fingerprints were identical in the majority of 35 patients studied, and where changes did occur they were random and minor (Thein et al., 1987). There is no indication that changes towards a common pattern might occur in culture.

The results on the three other lines tested indicate that they are of independent origin, confirming the isozyme analysis (Masters et al., 1986). The results using the Y-specific probe are also useful, as they suggest that the contaminating line was derived from a woman, as was T24, whereas the cross-contaminated lines were thought to be derived from men. Some of these lines contained chromosomes morphologically identified as Y-chromosomes (Hastings & Franks, 1983; Lin et al., 1985), presumably in error.

In conclusion, we regard these results as conclusive evidence that T24 and the five other lines in question were derived from the same individual. It is likely that probes such as these will superecede isozyme typing for the characterization of individuals and cell lines. Nevertheless, the guidelines have not changed. Firstly, when establishing a new cell line, normal tissue (usually blood cells) should be typed to confirm the identity of the culture. Secondly, cell lines should be typed at regular intervals to exclude cross-contamination.

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