

Assessment of DNA 'fingerprinting' as a method for validating the identity of cancer cell lines maintained in long-term culture

Martin F.Fey and Andreas Tobler[1,2]
Institute of Medical Oncology, Inselspital, [1]Institute of Clinical and Experimental Research, University of Berne, Tiefenauspital and [2]Central Haematology Laboratory, Inselspital, CH-3010 Berne, Switzerland

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DNA 'fingerprinting' with minisatellite probes has been suggested to be helpful for the individual-specific identification and authentication of cell lines and DNA 'fingerprints' have been reported to be stable over time (1–4). However, DNA 'fingerprints' of cancer cell lines may be unstable, since in cancers clonal somatic mutations are fairly frequent (5). We set out to see whether in leukaemia and lung cancer cell lines DNA 'fingerprints' would remain stable enough so as to permit their use for the validation of cell line identity after numerous continuous *in vitro* passages.

The human cancer cell lines HL-60 (6; promyelocytic leukaemia), S-LB1 (7; T-cell leukaemia) and LU-CSF-1 (8; lung adenocarcinoma) were maintained in continuous liquid culture for about one year up to 90 passages (P).DNA was extracted from cells taken at P +1 and at every 10th passage thereafter. DNA 'fingerprinting' (*Hinf*I, *Hae*III and *Alu*I digests) was done as described (5, 9). We used the minisatellite probes 33.15, 33.6 (1), pV47-2 (10) and α-globin 3′ hypervariable region (α-globin 3′HVR; 11).

In each of the three cancer cell lines the four minisatellite probes detected individual-specific DNA 'fingerprints' which differed for each probe. DNA 'fingerprints' visualized by the probes 33.15, 33.6 and pV47-2 did not change over time. However, in the two leukaemia cell lines DNA 'fingerprints' obtained with the α-globin 3′HVR probe did not remain stable at late passages. As of P +70 in S-LB1 cells and P +80 in HL-60 cells, novel bands and losses of bands were noted with all three enzymes. In each of these two cell lines, band sharing between DNA from late passages (≥ +70/+80, respectively) and earlier passages was reduced to 85–95% (Figure 1). In S-LB1 cells an unrelated clonal marker, biallelic TCR Cβ1 rearrangement (12), was stable in all the passages.

DNA 'fingerprints' detected by the probes 33.6, 33.15 and pV47-2 were suitable to validate the identity of cancer cell lines throughout the passages tested. Although the α-globin 3′HVR probe detected DNA 'fingerprint' instability in late passages from the leukaemic cell lines, band sharing between early and late passages of a given cell line was still in the order of 90% which is unlikely to occur by chance between two cell lines established from unrelated individuals. Therefore, individual-specific identification of cancer cell lines by DNA 'fingerprinting' should basically be reliable even at late passages (1). DNA 'fingerprinting' may thus be useful to trace back the origin of particular batches of cancer cell lines regardless of their passage number, and it may help to control for cross-contamination with cell lines derived from unrelated individuals.

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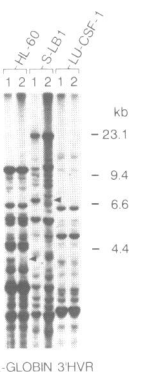

DNA 'fingerprints' in the cell lines HL-60 (1: P +20, 2: P +80), S-LB1 (1: P +10, 2: P +70) and LU-CSF-1 (1: P +40, 2: P +80). *Hinf*I digests, α-globin 3′HVR probe. Molecular size marker (*Hind*III digested λ phage DNA) indicated in kilobases. Arrows indicate deleted fragments in DNA from late passages of the two leukaemia cell lines.