A novel L1 retrotransposon marker for HeLa cell line identification

Raheleh Rahbari, Tom Sheahan, Vasileios Modes, Pam Collier, Catriona Macfarlane, and Richard M. Badge

Department of Genetics University of Leicester, Leicester, UK, and
Department of Cancer Studies and Molecular Medicine Leicester Royal Infirmary, Leicester, UK

Introduction

The history of the HeLa cell line is long and somewhat controversial (1). Despite its pivotal role as the first continuous human cancer cell line, the identity of the HeLa cell line has been accompanied by uncertainty. Although originally reported in 1952 as a cervical epithelial carcinoma from a female African-American individual, subsequent analysis of tumor pathology and clinical phenotype clearly identified the primary tumor as a rare adenocarcinoma (1). As the first human cell line to be widely cultured, HeLa has undergone strong adaptation to culture conditions. While karyotypically highly abnormal (4n = 82) and carrying cytologically distinct marker chromosomes (2), it is phenotypically epithelial in appearance, grows rapidly (doubling time is ~24 h), and shows a lack of contact inhibition (3). These characteristics and its ubiquity in cell culture laboratories have led to frequent cross-contamination. It has been estimated that 18% of cell lines submitted to repositories are contaminated with other cell lines, with HeLa alone being responsible for 25% of these cross-contamination events (2). In many cases, the originally deposited line has been completely replaced, resulting in the loss of potentially unique research resources in the very facilities designed to ensure their maintenance. There is also little doubt that cell line contamination in general—and HeLa contamination in particular—has led to the publication of erroneous research data (4), with HeLa sublines masquerading variously as amnion cells (WISH), embryonic lung cells (L132) and liver cells (Chang liver) (5).

The advent of robust and fairly inexpensive short tandem repeat (STR)–based DNA (STR DNA) fingerprinting technologies (6) has enabled the development of international reference standards for cell line identification. These standards, in principle, should eliminate cell line cross-contamination as a source of error in research using established cell lines, and should allow validation of provenance for newly derived human cell lines. The major cell line repositories (Coriell, ATCC, ECACC, DSMZ, JCRB) routinely use STR DNA fingerprinting techniques to monitor the status of their cell line stocks. However, part of the problem with the use of contaminated cell lines is the requirement for the regular and routine assay of cell line identity. It has been suggested that mandatory monitoring of cell line identity, as a condition for funding and/or publication (7), could eliminate cell line contamination. Meanwhile, in the absence of such requirements, the expense of STR-based monitoring to research laboratories is clearly a barrier to self-regulation. Given that the HeLa cell line is disproportionately responsible for cross-contamination events, a specific assay for this contamination could be included in cell culture practice, in much the same way as mycoplasma contamination can be routinely monitored by PCR (8). Here we present an assay that may remove some of these barriers.

L1 retrotransposons are active, autonomous mobile elements that are very common in the human genome [comprising 17% of the human genome sequence (9)]. Human-specific L1s (L1Hs) have been inserting into our DNA since the origin of the species and thus are readily employed as population markers with a number of useful characteristics. They are large (up to 7 kb) and stable insertions that undergo precise reversion only very rarely (12). During ongoing studies of human-specific, full-length, and potentially active L1 elements, we identified [using genome-wide transposon display methods (11)] a full-length L1 element that appeared to be HeLa-specific. Initial screening experiments showed that this insertion was present in a panel of widely geographically distributed HeLa isolates obtained from cell culture repositories in Europe and the United States, and absent from a large panel of human DNA samples. To fully realize the potential of this insertion as a HeLa-specific molecular diagnostic, we designed a single PCR-based genotyping assay that can be utilized in laboratories with access to standard molecular biology reagents and equipment. The assay is internally controlled, is sensitive to even low levels of HeLa contamination, and can be performed on unpurified cell pellets, allowing rapid routine screening.

Materials and methods

Tissue culture and DNA sources

The majority of HeLa and non-HeLa cell lines were obtained as gifts from collaborators (see Table 1). Where necessary, lines were revived from frozen storage and cultured at 37°C under 7% CO2 in low glucose DMEM (Gibco/Invitrogen, BioTechniques 46:277-284 (April 2009) doi 10.2144/000113089
Keywords: cell culture; LINE-1; HeLa; contamination; genotyping
DNA samples from anonymous black donors (from Harare, Zimbabwe) were provided by Sir Alec J. Jeffreys FRS (University of Leicester, Leicester, UK). Northern European DNA samples were obtained from the Centre d’Etude du Polymorphisme Humain (CEPH)/Fondation Jean Dausset. African, African-American, German Caucasian, Asian, and South American DNA samples were obtained from the Coriell Institute for Medical Research (Camden, NJ, USA).

DNA extraction
DNA was extracted from cultured cells using the Puregene Tissue Culture kit (Qiagen UK, Crawley, West Sussex, UK) following the manufacturer’s protocol. DNA integrity was assessed by fractionation on 0.8% (w/v) agarose gels [0.5× Tris-Borate-EDTA (TBE)] alongside intact bacteriophage λ DNA. DNA was visualized by ethidium bromide (0.5 μg/mL) staining under UV illumination.

PCR genotyping
Initial PCR genotyping assays used 1× PCR buffer (45 mM Tris-HCl pH 8.8, 11 mM NH₄SO₄, 0.9 mM MgCl₂), 6.7 mM β-mercaptoethanol, 113 μg/mL bovine serum albumin (BSA), 200 μM dNTPs, 0.05 U/μL Taq DNA polymerase (Abgene, Epsom, Surrey, UK) and primers [empty site: VM164A (5′-TGCCCTCTAGATCTGTATCC-3′) and VM164B (5′-GCACCTTGTGGAATGCAATTCA-3′); filled site: VM164A and RB5-PA2 (5′-TGGAAATGCAGAAATCCG-3′)]. PCR primers were synthesized by Sigma-Genosys/Aldrich (Gillingham, Dorset, UK). Ten to twenty nanograms of genomic DNA were added to 10-μL PCR reactions and cycled in a Tetrad 2 Thermal Cycler (MJ Research/Bio-Rad, Hercules, CA, USA). Cycle conditions were 30 cycles of 96°C for 30 s, 63°C for 30 s, 72°C for 1 min; then 1 cycle of 72°C for 5 min. Single-tube HeLa-specific PCR used 1× PCR buffer and primers VM164A, VM164B, and RB164K2 (5′-TGCTTTCTCGTACCC-3′). Cycle conditions were 30 cycles of 96°C for 30 s, 63°C for 30 s, 72°C for 1 min; then 1 cycle of 72°C for 5 min. PCR reactions were fractionated on 2% (w/v) agarose gels (0.5× TBE) and DNA was visualized by ethidium bromide (0.5 μg/mL) staining.

Dollopting
PCR-amplified DNA was denatured with 0.5M NaOH, 2M NaCl, 25 mM EDTA and transferred to nylon membranes using a 96-well dotblot vacuum manifold (BRL, Gaithersburg, MD, USA). DNA was covalently linked to the membrane by UV crosslinking (CL-1000 UV Crosslinker, UVP, Cambridge, UK). Radio-labeled empty site-specific probes were generated by labeling 25–50 ng of the empty site amplicon (amplified with primers VM164A/VM164B) with α-32P dCTP using the Amersham Rediprime II DNA labeling system (GE Healthcare, Fairfield, CT, USA) according to the manufacturer’s protocol. Hybridization was carried out overnight at 65°C in modified Church buffer (0.5 M NaH₂PO₄/Na₂HPO₄ pH 7.2, 7% SDS, 1 mM EDTA). Hybridized membranes were washed twice in 0.2× sodium chloride-sodium citrate (SSC) and 0.5% sodium dodecyl sulfate (SDS), and once in 0.1× SSC/0.1× SDS, at 65°C. Dotblots were visualized by autoradiography.
Results and discussion

As illustrated in Figure 1A, the AL137164 locus (chr14q12) in HeLa cells harbors a full-length human-specific L1 insertion, located in intron 2 of the STXBP6 (amisyn) gene. This L1 element is inserted in the same transcriptional orientation as the amisyn gene, has intact open reading frames (ORFs), and is likely to be active in cell culture retrotransposition assays (V. Modes and R. Badge, unpublished data). HeLa is triploid with respect to this otherwise karyotypically normal chromosome: PCR and direct sequencing of flanking markers showed that HeLa cells carry two empty site chromosomes (i.e., that lack the L1 insertion) and one filled site chromosome (data not shown). All tested HeLa isolates were heterozygous for the insertion (i.e., they contain at least one empty and one filled site chromosome).

To establish the population frequency of this L1 insertion, and to determine if this marker could be used unequivocally to identify HeLa and HeLa-derived cell lines, we applied locus-specific genotyping assays to diverse human DNA samples. Samples from 72 unrelated black Zimbabwean individuals were genotyped (of DNA we have tested, this sample set is most closely related to the HeLa donor’s likely population of origin), using a sensitive dotblot assay. In this assay PCR amplicons specific for the empty site (chromosomes lacking the L1 insertion) site and filled site (chromosomes harboring the L1 insertion) were hybridized with a radiolabeled probe derived from the empty site, as shown in Figure 1B. This probe detects not only the empty site amplicon, but also the filled site, due to the presence of 3′ flanking genomic sequence in the amplicon. All tested Zimbabweans were homozygous for the insertion empty site. Genotyping 364 unrelated individuals from geographically diverse populations (Africans, African-Americans, Northern Europeans, German Caucasians, Asians, and South Americans) failed to identify any other carriers of the AL137164 L1 insertion (data not shown).

The original genotyping assay was modified into a single duplex PCR format (Figure 2A), providing a simple way to discriminate HeLa and non-HeLa cell lines. As shown in Figure 2B, all tested HeLa isolates, including the HeLa S3 subline, were positive using this assay (they all generated a 325-bp insertion–specific amplicon), and 11 tested non-HeLa lines were negative (they lacked this filled site–specific amplicon).
Figure 3. The duplex PCR assay can detect low-level HeLa contamination and can be applied to unpurified cell pellets. (A) The genotyping assay can detect low-level HeLa DNA contamination. Genomic DNA from the HeLa and Ntera2D1 cell lines was mixed in varying ratios and subjected to PCR amplification in triplicate, using the three-primer duplex genotyping assay. The HeLa-specific AL137164 amplicon is detectable at a ratio of 1% HeLa DNA to 99% Ntera2D1 DNA. M, 100-bp ladder molecular weight marker; Lanes 1–3, 100% HeLa genomic DNA (20 ng/reaction); Lanes 4–6, 50% HeLa, 50% Ntera2D1; Lanes 7–9, 50% HeLa, 50% Ntera2D1; Lanes 10–12, 99% Ntera2D1; Lanes 13–15, 1% HeLa, 99% Ntera2D1; Lane 16, PCR negative control (no input DNA). (B) Unpurified frozen cell pellets are suitable genotyping substrates. Frozen cell pellets (∼1 × 10^6 cells/mL) from HeLa and non-HeLa cell cultures were defrosted, diluted in 5 mM Tris-HCl, pH 7.0 and boiled for 5 min. The boiled extracts were amplified using the three-primer duplex genotyping assay. M, 100-bp ladder molecular weight marker; Lane 1, input 50% HeLa cell pellet, 50% 5 mM Tris-HCl, pH 7.0; Lane 2, input 10% HeLa cell pellet, 50% 5 mM Tris-HCl, pH 7.0; Lane 3, 5% HeLa, 95% 5 mM Tris-HCl, pH 7.0; Lane 4, 2% HeLa, 98% 5 mM Tris-HCl, pH 7.0; Lane 5, 1% HeLa cell pellet, 96% 5 mM Tris-HCl, pH 7.0; Lane 6, input 50% non-HeLa cell pellet, 50% 5 mM Tris-HCl, pH 7.0; Lane 7, 10% non-HeLa cell pellet, 90% 5 mM Tris-HCl, pH 7.0; Lane 8, 5% non-HeLa cell pellet, 95% 5 mM Tris-HCl, pH 7.0; Lane 9, 2% non-HeLa cell pellet, 98% 5 mM Tris-HCl, pH 7.0; Lane 10, 1% Non-HeLa cell pellet, 99% 5 mM Tris-HCl, pH 7.0; Lane 11, PCR positive control (10 ng HeLa gDNA); Lane 12, PCR negative control (no input DNA). The gradient triangles indicate the proportion of cell pellet extract in the input to the genotyping PCR.

DNA mixing experiments (Figure 3A) using HeLa DNA and Ntera2D1 genomic DNA indicate that the single-tube assay is highly sensitive, and can detect at least 1% of HeLa DNA. The assay is also robust with respect to the input material: even genomic DNA present in unpurified frozen cell pellets (Figure 3B) can be genotyped using this assay.

It is evident that the HeLa cell line is responsible for a disproportionate number of cell line contamination events, likely in part due to its unusual origin as an aggressive cervical adenocarcinoma, as well as an adaptation to cell culture over its long history (1). Despite the availability of STR-based DNA fingerprinting technology to unequivocally identify cell lines (6), contaminated cell lines are still used erroneously in peer-reviewed publications (4). One reason for this may be ignorance of the status of these lines, but the expense of routine cell line identification monitoring may also play a role in the persistence of these compromised experimental resources. Even vigilant monitoring of cell line identity by cell culture stock centers cannot guard against contamination events in individual laboratories. The widespread sharing of cell line cultures between laboratories and their resultant poor provenance—although pivotal in enabling this particular study—will hinder a solution to this problem. Recent calls to action on this issue (17,18) as well as assessments of the costs of using unauthenticated cell lines in research (7), show that the need to develop inexpensive, rapid and sensitive cell identity assays is topical and pressing.

Having fortuitously discovered what appears to be a HeLa-specific full-length L1 insertion, we screened donated HeLa cell isolates and found that independently acquired and geographically dispersed HeLa isolates all share this marker. To establish whether this marker might occur in other human cell lines, we determined the population frequency of the insertion in a diverse panel of human DNA samples. The insertion could not be located in a panel of 72 DNA samples from Zimbabwean donors, despite this population sample being closely related to the HeLa donor’s likely origin (of the DNA we have tested) and the large proportion of worldwide human genetic diversity preserved in African populations. Given the tiny proportion of human genomes that have been immortalized as cell lines, it therefore seems most likely that this L1 insertion is specific for and unique to the HeLa cell line.

To improve the utility of this marker system, we adapted the genotyping assay to a single PCR. We also showed that the assay is sensitive to small amounts of contaminating cell line DNA, and that it works reliably with frozen cell pellets, a resource that is routinely available in cell culture laboratories.

In conclusion, we present a simple, rapid, and robust assay for detecting HeLa, the most common contaminating cell line. As it is PCR-based, insensitive to DNA condition and purity, and highly specific for HeLa, this assay should be accessible to any lab performing routine molecular biology. As such, it removes at least one barrier (that of cost) to routine cell line monitoring, and may contribute to the elimination of erroneous research based on contaminated or false cell lines.

Acknowledgments

The following colleagues kindly provided aliquots of HeLa, HeLa-derived, and control cell lines for analysis: Nicola Royde (University of Leicester), Andrew Fry (University of Leicester), Raj Patel (University of Leicester), Simon Kilvington (University of Leicester), Fred Tata (University of Leicester), John Moran (University of Michigan), Fred Gage (Salk Institute), Zimbabwean DNA samples were kindly made available by Professor Sir Alec J. Jeffreys FRS (University of Leicester). The research...
was funded by the Wellcome Trust (grant no. 075163/Z/04/Z) to R.M.B and A.J.J.

The authors declare no competing interests.

Reference


Received 15 September 2008; accepted 22 December 2008.