Published in final edited form as:
Gynecol Oncol. 2012 October ; 127(1): 241–248. doi:10.1016/j.ygyno.2012.06.017.

DNA profiling analysis of endometrial and ovarian cell lines reveals misidentification, redundancy and contamination

Christopher Korch[a,1], **Monique A. Spillman**[b,1], **Twila A. Jackson**[b,1], **Britta M. Jacobsen**[c], **Susan K. Murphy**[d], **Bruce A. Lessey**[e], **V. Craig Jordan**[f], and **Andrew P. Bradford**[b,*]

[a]Division of Medical Oncology, University of Colorado Anschutz Medical Campus, Aurora, CO 80045

[b]Department of Obstetrics & Gynecology, University of Colorado Anschutz Medical Campus, Aurora, CO 80045

[c]Department of Medicine, Division of Endocrinology, University of Colorado Anschutz Medical Campus, Aurora, CO 80045

[d]Department of Obstetrics & Gynecology, Duke University Medical Center, Durham, NC 27708

[e]Division of Reproductive Endocrinology and Infertility, Greenville Hospital System, Greenville, SC 29605

[f]Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC 20057

Abstract

Objectives—Cell lines derived from human ovarian and endometrial cancers, and their immortalized non-malignant counterparts, are critical tools to investigate and characterize molecular mechanisms underlying gynecologic tumorigenesis, and facilitate development of novel therapeutics. To determine the extent of misidentification, contamination and redundancy, with evident consequences for the validity of research based upon these models, we undertook a systematic analysis and cataloging of endometrial and ovarian cell lines.

Methods—Profiling of cell lines by analysis of DNA microsatellite short tandem repeats (STR), p53 nucleotide polymorphisms and microsatellite instability.

Results—Fifty-one ovarian cancer lines were profiled with ten found to be redundant and five (A2008, OV2008, C13, SK-OV-4 and SK-OV-6) identified as cervical cancer cells. Ten endometrial cell lines were analyzed, with RL-92, HEC-1A, HEC-1B, HEC-50, KLE, and AN3CA all exhibiting unique, uncontaminated STR profiles. Multiple variants of Ishikawa and ECC-1 endometrial cancer cell lines were genotyped and analyzed by sequencing of mutations in the p53 gene. The profile of ECC-1 cells did not match the EnCa-101 tumor, from which it was reportedly derived, and all ECC-1 isolates genotyped as Ishikawa cells, MCF-7 breast cancer cells,

*Corresponding author at: University of Colorado, Anschutz Medical Campus, Department of Obstetrics & Gynecology, MS 8613, 12700 E. 19th Avenue, Aurora, CO 80045, Tel: (303) 724 3507, Fax: (303 724 3512, Andy.Bradford@ucdenver.edu.
[1]joint first authors

Conflict of Interest statement
No conflict of interest

or a combination thereof. Two normal, immortalized endometrial epithelial cell lines, HES cells and the hTERT-EEC line, were identified as HeLa cervical carcinoma and MCF-7 breast cancer cells, respectively.

Conclusions—Results demonstrate significant misidentification, duplication, and loss of integrity of endometrial and ovarian cancer cell lines. Authentication by STR DNA profiling is a simple and economical method to verify and validate studies undertaken with these models.

Keywords

STR profiling; Endometrial; Ovarian; cell lines; authenticity

Introduction

Cell lines, immortalized from normal human tissues or derived from tumors, are widely used models to address molecular mechanisms underlying the physiology and pathology of the female reproductive tract, and to evaluate novel therapeutics or preventive strategies [1–3]. Verification of the provenance and integrity of such cell lines is clearly of paramount importance, but historically, has rarely been undertaken by investigators. The problem of cross-contamination, identified and characterized by examination of isozyme patterns, karyotyping, and cytogenetics, dates back to the establishment of the prototypical HeLa cell line in culture in 1951 and remains a significant concern [4–7]. Over one-third (18–50%) of cell lines may be mixtures, misidentified or intra-species contaminants [2, 8–15]. Furthermore, there are many examples of redundancy among reportedly unique cell lines, and instances of contamination during original derivations, such that the intended novel cell line was never established [5, 10, 16–19]. Thus, it is evident that authentication of cell line origins and integrity is crucial to validate results and conclusions obtained using these model systems.

Short tandem repeat (STR) profiling or ‘DNA fingerprinting’ identifies variants in tetranucleotide microsatellite loci on multiple human chromosomes and is the accepted international standard for genetic analysis of cell lines for authentication by comparison to established STR databases [20–24].

A comprehensive analysis of cell lines commonly used in the study of ovarian and endometrial cancer had not been undertaken, particularly with respect to those cell lines not obtained from established cell repositories. We used STR profiling, sequencing of p53 mutations, and human papilloma virus screening to examine cell lines of purported ovarian and endometrial origins. We observed examples of cross-contamination, misidentification of lines and/or tissue of origin, and redundancy among established cancer cells, and found evidence that immortalized normal endometrial epithelial cell lines are genetically identical to previously established cervical and breast cancer cells. We provide reference DNA profiles for women's cancer cell lines that are not currently in public cell banks and extend the number of loci for profiles currently available through central repositories.

Materials and methods

DNA isolation and STR profiling

Cell lines were grown in appropriate specific standard media. Genomic DNA was isolated from 0.5–5 × 10^6 cells using a Zymo Research ZR genomic DNA II kit and quantified by gel electrophoresis and ethidium bromide staining by comparison to a DNA mass ladder. Multiplex PCR amplified products were generated using 1–2 ng of genomic DNA with an Applied Biosystems Identifiler kit and ABI 3730 capillary sequencer as described [2, 18]. STR loci were analyzed with Gene Mapper 4.0. Profiles were compared to published reports

[22, 25], consolidated (ATCC, DSMZ, JCRB and RIKEN) databases, and an in-house database, using a custom search algorithm designed to facilitate comparison of cell lines with related profiles and identify individual cell lines in a mixture (C. Korch and J. West, Vanderbilt University, unpublished). STR profiles of the ovarian and endometrial cancer cells analyzed in this study are available online at http://DNAsequencingcore.UCDenver.edu.

TP53 sequence analysis and microsatellite instability assays

PCR amplification was used to generate overlapping products spanning the Variable Number Tandem Repeat (VNTR; a pentanucleotide repeat of A_4T) in intron 1, through the protein encoding exons 2–11, including intervening introns 2–8 and 10 [26]. Sequencing primers and p53 gene structure are shown in Figure S1. DNAs were screened for microsatellite instability [27] using Promega MSI analysis system version 1.2 according to the manufacturers protocol.

HPV testing

Aliquots of cells were placed into ThinPrep (Hologic) solution. DNA was isolated and tested in University of Colorado Hospital Clinical Laboratory using the hybrid capture PCR, Digene HC2 High Risk HPV test (Qiagen).

Ovarian and endometrial cell lines

We obtained cell lines from multiple institutions in the United States, Europe and Japan, including, where possible, the originating laboratories. Multiple independent samples of the earliest available passages from each institution were analyzed and, if available, profiles of each individual cell line were compared from several sources. Ovarian cancer cell lines are listed in Table S1. Ishikawa cells were obtained from Dr. K.K. Leslie (University of Iowa), Dr. B.A. Lessey (Greenville Hospital System, SC), Dr. M. Brown (Dana Farber Cancer Institute, Harvard University) and Drs H. Philpott and P. Thraves (European Collection of Cell Cultures, ECACC). ECC-1 cells were from Drs. B.A. Lessey, M. Brown and V.C. Jordan (Lombardi Comprehensive Cancer Center, Georgetown University). EnCa-101 tumors were provided by Drs. V.C. Jordan and G. Balburski (Fox Chase Cancer Center). HES cells were from Dr. D. Kniss (Ohio State University) and hTERT-EECs from Dr. T. Klonisch (University of Manitoba, Canada). KLE and HEC-50 cells were from Dr. K.K. Leslie. RL-95-2, HEC-1A, HEC-1B and AN3CA cells were from the American Type Culture Collection (ATCC, Manassas, VA).

Results

Analysis of endometrial cancer cell lines

Endometrial carcinomas are derived from glandular epithelium and are typically divided into two subtypes based on clinical, histological and molecular characteristics [28–30]. Cell lines derived from type I (Ishikawa, ECC-1 and RL-95-2) and type II (HEC-1, HEC-50, KLE and AN3CA) tumors have been widely used as models to investigate molecular genetics and mechanisms underlying their development, progression and response to therapeutics [31–35].

HEC-1B cell lines, the first to be derived from a human endometrial carcinoma [32, 36, 37], both exhibited a unique profile(Table S3). HEC-1A cells are predominantly diploid, while the HEC-1B line is tetraploid [38, 39]. HEC-50 cells [38, 40], also have a unique profile consistent with that on file with the Japanese Collection of Research Bioresources (JCRB: 1145).

Similarly, KLE (CRL-1622) and AN3CA (HTB-111) cells, originating from peritoneal and lymph node metastases, respectively [34, 41, 42], and RL-95-2 cells (CRL-1671) derived from a moderately differentiated (Grade 2) endometrial adenosquamous carcinoma [35], all have STR profiles consistent with those reported by the ATCC (Table S3).

Ishikawa cells were established from the epithelial component of a moderately differentiated, stage 2, endometrial adenocarcinoma [43, 44]. At least three variants of Ishikawa cells, the original line, 3-H-4 and 3-H-12, differing in their reported degree of differentiation, relative expression of estrogen (ER) and progesterone (PR) receptors, growth and colony formation rates, were distributed to investigators [45].

We profiled multiple isolates of the original Ishikawa cells and 3-H-12 variants obtained from a number of laboratories as detailed in Materials and Methods. Samples with unique profiles, which may represent the 3-H-4 variant based upon their date of origin are designated ‘3-H-4’. The results are summarized in Table 1.

Overall the Ishikawa cell lines exhibit very similar profiles, indicative of their originating from the same patient. Identical alleles were present at several loci (CSF1PO, D5S818, D16S539, D21S11, THO1 and TPOX). Others reflect loss or gain of alleles (D8S1179, D13S317 and FGA) or alterations in the number of repeats (D2S1338, D3S1358, D19S433 and vWA). At the D7S820 locus, the original Ishikawa isolate exhibits 8.3- and 11-repeat alleles, whilst subsequent sublines display 9- or 10-repeats. The D18S51 locus was found to be highly polymorphic in most Ishikawa lines.

Minor differences in the number of repeats at certain loci are consistent with the known microsatellite instability (MSI) of these lines, due to mutations in mismatch repair systems [46–48], and suggest that these variants arose by genetic drift between different clonal isolates over hundreds of cell passages. Accordingly, all Ishikawa cell lines exhibited high variability/ instability at microsatellite loci (Table S2). Defective mismatch repair also underlies allelic variation in AN3CA cells (Table S3) [49]. In contrast, EnCa-101 tumors and MCF-7 cells were MSI stable.

We also profiled a variant of Ishikawa cells lacking ER [50]. Previous reports implied that these cells, also known as Ishikawa B, were derived from a different patient [51, 52]. The STR profile of ER-negative Ishikawa cells exhibits minor variations from other Ishikawa sublines (Table 1), but overlap at the majority of loci indicates a common origin.

A second type 1, ER and PR positive cell line, ECC-1, was established from a grade 2, well-differentiated, endometrial carcinoma adenocarcinoma [42, 53, 54]. The line was derived by passage of the tumor, designated EnCa-101, in nude mice and subsequent isolation of PR positive cells from an epithelial monolayer culture [42, 55]. ECC-1 cells were described as a well-differentiated, steroid responsive line with a phenotype characteristic of luminal surface epithelium, distinct from Ishikawa cells, which expressed markers of glandular endometrial epithelium [33].

Upon STR and MSI analysis, ECC-1 samples exhibited DNA profiles essentially identical to Ishikawa 3-H-12 cells (Tables 1 & S2). In addition, the ATCC profile for ECC-1 also closely matched that of earlier Ishikawa cells on file with the European Collection of Cell Cultures (ECACC). Other ‘ECC-1’ cell lines were found to be identical to MCF-7 breast cancer cells or consist of a mixture of Ishikawa and MCF-7 cells (not shown). Unfortunately, following the death of Dr. Satyaswaroop, records and cell lines from his laboratory were lost or destroyed (Zaino, R. & Lessey, B., personal communication). Thus, we could not obtain reference samples of the original ECC-1 line or EnCa-101 tumor from which it was purportedly derived. However, the EnCa-101 tumor has been continuously

maintained in mice [56] and we obtained and analyzed 3 independent samples. Profiling of these tumors showed minor variations, but results indicated they were derived from the same human patient. In contrast, the unique EnCa-101 profiles did not match ECC-1, Ishikawa or MCF-7 cell lines (Table 1). These data are inconsistent with the reported origins of ECC-1 cells and suggest that the original line has been lost. Our results show that currently available ECC-1 cells are Ishikawa cells, MCF-7 breast cancer cells, or a mixture of both.

Sequencing of p53 mutations in endometrial cancer cells

To confirm the apparent equivalence of Ishikawa and ECC-1 cells, we screened for p53 mutations by PCR amplification and sequencing of the Variable Number Tandem Repeat (VNTR) region in intron 1, and the protein encoding exons and introns (Fig. S1). Table 2 lists the observed p53 mutations and SNPs compared to the reference/ normal sequence.

In agreement with previous reports [31, 57], Ishikawa original and 3-H-12 cells harbor a Met 246 Val mutation in exon 7. These two lines are also homozygous in the VNTR region with 8 repeats of A_4T, heterozygous in exon 4 for the Asp 49 Val mutation (nucleotide G12069S), and heterozygous in intron 10 for deletion of the seventh T in a heptanucleotide repeat (17822delT). The original Ishikawa sample has two additional heterozygous mutations, 12724insA (intron 4) and 13764delA (intron 6), which are not present in the 3-H-12 line (Table 2).

Possible ‘3-H-4’ sublines have a similar profile, but lack the intronic 12724insA and 13764delA mutations of poly A stretches, present in the original Ishikawa lines (Table 2). An additional heterozygous mutation in intron 4 (G12299K (G+T)) was detected in some Ishikawa 3-H-12 sublines. Interestingly, consistent with their closely matched STR profiles, the ER-negative Ishikawa cells, despite their purported distinct origin, exhibit TP53 mutations identical to Ishikawa 3-H-12 and ‘3-H-4’ (not shown). TP53 mutations unique to the original Ishikawa lines are insertions or deletions in homopolymer A or T stretches, which are consistent with microsatellite instability due to mutations in the mismatch repair system [46].

In agreement with their identical STR profiles, ECC-1 cells show the same TP53 mutations as Ishikawa 3-H-12 lines, further evidence that ECC-1 are misidentified Ishikawa cells. In contrast, EnCa-101 tumors have completely different TP53 mutations from the Ishikawa and ECC-1 lines (Table 2), again demonstrating that ECC-1 cells are not derived from the EnCa-101 tumor. ‘ECC-1’ cells shown to be contaminated with or identical to MCF-7 cells were not subjected to TP53 analysis.

Finally, our data suggest that only one copy of the p53 gene is expressed in Ishikawa cells. In the genomic DNA, both the A14063R (A+G) and G12069S (G+C) positions are heterozygous. However, only the 14063G mutation is present in the cDNA sequence [31, 57], suggesting that the G12069C mutation is in the unexpressed copy of the gene.

Analysis of normal endometrial epithelial cells

Immortalized, non-transformed endometrial epithelial cells are a potentially valuable resource to investigate normal uterine physiology and tumorigenesis. We profiled two such lines, human endometrial (HES) cells [58] and hTERT-EEC [59], obtained from their developers, which have been extensively used as models of normal endometrium. Neither cell line was authenticated as they exhibited DNA profiles corresponding to HeLa and MCF-7 cancer cells, respectively.

HES cells were established, in 1989, from a primary culture of benign proliferative endometrium, which apparently underwent spontaneous transformation after serial passage

[58, 60]. Profiling of these cells (Table 3) indicated they are identical at all loci to HeLa cervical carcinoma cells, specifically the HeLaS3 variant. HES cells are also identical to WISH cells, a cell line originally described as derived from human amnion [61] but subsequently also identified as HeLa [7, 62, 63]. These results were independently confirmed by the STR fragment analysis facility at Johns Hopkins University (D. Kniss, Ohio State University; personal communication).

hTERT-EECs were isolated from normal proliferative phase endometrial epithelium and immortalized by stable transfection with the catalytic subunit of human telomerase (hTERT) [59]. Replicate STR profiling of the earliest available passages of multiple clonal lines indicated all isolates of hTERT-EEC cells to be genetically identical to MCF-7 breast cancer cells (Table 3). As for HES cells, this was not attributable to contamination as no other profiles were detected in the samples.

Analysis of ovarian cancer cell lines

We obtained and genotyped fifty-one ovarian cancer cell lines (Table S1), many of which are not available from public repositories. Two of the lines (IGROV1 and OVCAR-10) gave mixed genotypes indicating cross-contamination and were excluded from further analysis. The mixed genotype for IGROV1 was confirmed in multiple isolates including those obtained directly from the National Cancer Institute.

Several purported ‘ovarian cancer’ lines were genotypically identical to other known, non-ovarian, cancer cells: BG-1[64] was identified as MCF-7 breast cancer cells, and CH1, CH1cisR, and 222 as the teratocarcinoma line PA1. C13, A2008 and OV2008 were identical to the ME-180 (ATCC: HTB-33) cervical cancer cell line, and confirmed to be HPV positive (Table 4). The genotypically distinct 2008 cell line [65], obtained directly from the originating laboratory of Dr. Peter Disaia [66], was HPV negative. Finally, SK-OV-4 and SK-OV-6 lines matched HPV-negative C-33A (HTB-31) cervical cancer cells (Table 4).

Two ‘normal ovarian’ cell lines, NOSE06 and NOSE07, were genotyped as the ovarian cancer line DOV-13. Similarly, Caov-2 was identical to the earlier NIH:OVCAR-2 line (Table S4) and some samples of COLO-720E were found to be COLO-704 (not shown). Ovary1847 cells genotyped as NIH:OVCAR-8.

The remaining ovarian cancer cell lines exhibited unique, uncontaminated genotypes and are listed with their STR profiles in Table S4.

We noted disparate genotypes for several cell lines with similar names; 2008 cells are distinct from A2008 and OV2008, and 167 differs from OV167 cells. In contrast, the TOV-112D cell line is identical to TOV-21D, which appears to have arisen via transposition of numbers and letters in the name. Some isolates of TOV-112D were misidentified and matched TOV-21G cells.

The heterogeneity of ovarian tumor cells in ascitic fluid has previously lead to the establishment of several cell lines with different phenotypic characteristics [67]. We profiled very early passages of OV429 and OV433 [68, 69] and found identical genotypes, indicative of either a common patient origin or early cross-contamination (Table S4). Of historical note, OV433 was the cell line used originally to select for reactivity to the OC125 monoclonal antibody to the ovarian tumor marker CA125.

The cluster of PEO1/PEO4/PEO6 cells are known to originate from the same patient [70], and genotype accordingly. Similarly, HEY/HEYA8/HEYC2 cells [71] are derived from the same original line, and share identical genotypes (Table S4).

Chemotherapy resistant derivatives mirror parental cell line genotypes

We tested five original and cisplatin-resistant paired cell lines and all five parent and derivative combinations were confirmed by genotyping. However, as shown earlier (Table 4), the OV2008/C13 cells are cervical, not ovarian cancer cells and the CH1/CH1cisR lines [72] are PA1 teratocarcinoma cells. Table S5 shows STR profiles of the matched cisplatin-sensitive/ -resistant ovarian cancer cell lines. The 41M/41McisR, TYKnu/TYKnucisR and A2780/A2780cisR pairs each have unique profiles. The paired lines demonstrate some genetic instability, consistent with cisplatin-induced MSI [73]. Cisplatin-resistant A2780 cells have lost alleles at the D3S1358, FGA, D8S1179. D5S818, D7S820, CSF1PO, and D2S1338 loci, and gained an allele at the D18S51 locus. The 41M/41McisR pair is more stable, with the cisplatin-resistant line differing only at the vWA locus. The original derivation of the 41M cisplatin-resistant lines lists three isolates (41McisR2, 41McisR4 and 41McisR6), which differed in their IC_{50} [74]. The subline profiled herein is unknown, as the identifying number has been lost.

Discussion

Gynecologic cancer research is critically dependent on the use of cell culture models, to investigate molecular mechanisms underlying the development and progression of tumors, to design and test novel therapeutic strategies, and identify potential diagnostic or prognostic markers. In this report, we profiled the most widely used endometrial and ovarian cell lines and discovered several examples of misidentification, redundancy and cross-contamination.

Genotyping and HPV testing of ovarian cancer cell lines identified eight (BG-1 [64], CH1/CH1cisR [72], 222 [75], C13 [76], A2008 [77, 78], OV2008, SKOV-4 and SKOV-6 [79]) as previously existing, breast cancer, teratocarcinoma or cervical cancer cell lines. In addition, two ‘normal ovarian’ cell lines, NOSE06 and NOSE07 [80], were genotyped as the ovarian cancer line DOV-13 [81]. We also highlight the possibility for confusion of several ovarian cancer cell lines with similar names, but distinct genotypes; e.g. 167 and OV167, 2008 and A2008 /OV2008.

We profiled a number of variants of Ishikawa endometrial cancer cells. Results are consistent with a common origin for these sublines, with variations and polymorphisms in some STR loci attributable to genetic instability, mismatch repair defects, and high passage number [75–77]. Analyses of mutations in the p53 gene (TP53) are consistent with previous reports [31, 57] and provide additional genetic markers to perhaps distinguish the original, 3-H-4 and 3-H-12 Ishikawa lines. Furthermore, STR profiling, TP53 sequencing, and MSI analysis confirm that currently available isolates of ECC-1 cells are not authentic but are identical to Ishikawa cells, specifically the 3-H-12 line. This conclusion is reinforced by evidence that the EnCa-101 tumor, from which the original EEC-1 line was purportedly derived [42, 55], is genetically distinct from both Ishikawa and ECC-1 cells. We also observed several ECC-1 isolates to be misidentified MCF-7 cells or a cross-contaminated mixture of Ishikawa and MCF-7 lines.

ECC-1 cells were initially characterized as distinct from Ishikawa lines based on differential expression of cytokeratin 13 and osteopontin [33]. However, both markers were present in the two lines, which otherwise showed identical patterns of expression of steroid hormone receptors and their coactivators [33]. The karyotypes of Ishikawa and ECC-1 cells also exhibit some apparent differences [31, 33], but chromosomal number and structural rearrangements in both lines were complex with high intercellular variability [31, 33]. Comparative cytogenetic analysis found that, given the evident heterogeneity and differential capabilities of the techniques used (FISH or SKY) to detect abnormalities in

small chromosomal segments, the karyotypic similarity was likely underestimated, and is consistent with the two lines sharing a common origin.

Thus, we conclude that the original ECC-1 cell line has been lost, although the persistence of the EnCa-101 tumor [56] provides an opportunity for its re-derivation. ECC-1 cells have been extensively used as models of ER positive, type 1, endometrial cancers. Since Ishikawa cells are also representative of such endometrioid tumors, our evidence that the two lines are identical may not significantly impact conclusions drawn from these studies, beyond the use of two redundant cell lines. However, the possible misidentification of MCF-7 breast cancer cells as ECC-1, or cross contamination with the former, should be considered in interpreting results using ECC-1 cells.

We identified the normal endometrial epithelial cell line (HES) as HeLa cervical carcinoma cells. HES cells have been used as a model of benign endometrial epithelium to study mucosal immunity [82], implantation [83, 84], decidualization [85] and endometriosis [86], and have served as ‘normal’ controls for novel chemotherapeutics [87, 88] and analysis of signaling pathways in the endometrium [89–93]. Similarly, the telomerase immortalized endometrial epithelial cell line, hTERT-EEC [59], was an exact genotypic match to MCF-7 breast cancer cells. hTERT-EEC have been proposed as model to study steroids in normal endometrial physiology, including, endometriosis and implantation [59, 94, 95]. Clearly, conclusions derived from studies utilizing HES cells (HeLa) or hTERT-EEC (MCF-7) should be interpreted with caution, in the light of evidence that they are neither normal nor endometrial in origin.

Cell line authentication is essential for their meaningful use in research. We recommend that cell lines be quarantined and authenticated by DNA profiling prior to use, and periodically evaluated by STR genotype, to check for cross-contamination and validate construction of stably transfected, genetically modified or clonally selected variants. Derivation of novel cell lines should be accompanied, where possible, by STR profiles of the patient germ line, tumor or tissue, and cell line DNA. We also suggest use of histological or phenotypic markers to verify the tissue of origin, since STR profiling cannot provide this information resulting in debate as to the tissue type of some cancer cell lines [2, 96].

The origins and mechanisms of cell line contamination, including poor tissue culture technique, inadequate quality control, clerical and labeling errors, and aerosol transfer of cells, have been reviewed previously [63] and, despite best laboratory practices, are probably unavoidable. Accordingly, even among cell lines that exhibited unique profiles, we found examples, from all sources, of individual aliquots that were misidentified or contaminated, indicating a widespread and pervasive problem. STR profiling is a simple, widely available and relatively inexpensive method to document and authenticate cell lines, and has been recommended as an internationally accepted standard for human cells [22, 63, 97, 98]. Despite repeated calls for journals to require DNA profiling of cells for publication, this practice has not been widely adopted [63, 99]. Complacency and denial of the existence and extent of the problem with validation and authenticity of cell lines, while prevalent [7, 24, 63, 99], are antithetical to the conduct of responsible research in gynecologic oncology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to the investigators who generously provided cell lines for analysis. STR profiling and sequence analysis was carried out in the University of Colorado Cancer Center (UCCC) DNA Sequencing & Analyses Core.

We thank Dr. Marileila Varella Garcia of the UCCC Molecular Pathology Shared Resource Cytogenetics Core (NCI-P30CA046934) for karyotype comparison and analysis and Jerry Haney for MSI assays. This work was funded in part by NCI CA125427, NICHD HD067721, Lombardi Comprehensive Cancer Center Support Grant NIH P30 CA051008, Department of Defense grant W81XWH-11-1-0469, Cancer League of Colorado, Ovarian Cancer Research Fund, Foundation for Women's Cancer, Adelson Family Cancer Foundation and University of Colorado Department of Obstetrics and Gynecology.

References

1. Burdall SE, Hanby AM, Lansdown MR, Speirs V. Breast cancer cell lines: friend or foe? Breast Cancer Res. 2003; 5:89–95. [PubMed: 12631387]
2. Schweppe RE, Klopper JP, Korch C, Pugazhenthi U, Benezra M, Knauf JA, Fagin JA, Marlow LA, Copland JA, Smallridge RC, Haugen BR. Deoxyribonucleic acid profiling analysis of 40 human thyroid cancer cell lines reveals cross-contamination resulting in cell line redundancy and misidentification. J Clin Endocrinol Metab. 2008; 93:4331–41. [PubMed: 18713817]
3. Vollmer G. Endometrial cancer: experimental models useful for studies on molecular aspects of endometrial cancer and carcinogenesis. Endocr Relat Cancer. 2003; 10:23–42. [PubMed: 12653669]
4. Lucey BP, Nelson-Rees WA, Hutchins GM. Henrietta Lacks, HeLa cells, and cell culture contamination. Arch Pathol Lab Med. 2009; 133:1463–7. [PubMed: 19722756]
5. MacLeod RA, Dirks WG, Matsuo Y, Kaufmann M, Milch H, Drexler HG. Widespread intraspecies cross-contamination of human tumor cell lines arising at source. Int J Cancer. 1999; 83:555–63. [PubMed: 10508494]
6. McCormick D. Sleeper cell. Biotechniques. 2008; 45:11. [PubMed: 18683356]
7. Masters JR. HeLa cells 50 years on: the good, the bad and the ugly. Nat Rev Cancer. 2002; 2:315–9. [PubMed: 12001993]
8. Identity crisis. Nature. 2009; 457:935–6.
9. Chatterjee R. Cell biology. When 60 lines don't add up. Science. 2007; 315:929. [PubMed: 17303730]
10. Chatterjee R. Cell biology. Cases of mistaken identity. Science. 2007; 315:928–31. [PubMed: 17303729]
11. Freshney RI. Authentication of cell lines: ignore at your peril! Expert Rev Anticancer Ther. 2008; 8:311–4. [PubMed: 18366279]
12. Hughes P, Marshall D, Reid Y, Parkes H, Gelber C. The costs of using unauthenticated, over-passaged cell lines: how much more data do we need? Biotechniques. 2007; 43:575, 577–8, 581–2. passim. [PubMed: 18072586]
13. Nelson-Rees WA, Daniels DW, Flandermeyer RR. Cross-contamination of cells in culture. Science. 1981; 212:446–52. [PubMed: 6451928]
14. Rae JM, Creighton CJ, Meck JM, Haddad BR, Johnson MD. MDA-MB-435 cells are derived from M14 melanoma cells--a loss for breast cancer, but a boon for melanoma research. Breast Cancer Res Treat. 2007; 104:13–9. [PubMed: 17004106]
15. Capes-Davis A, Theodosopoulos G, Atkin I, Drexler HG, Kohara A, MacLeod RA, Masters JR, Nakamura Y, Reid YA, Reddel RR, Freshney RI. Check your cultures! A list of cross-contaminated or misidentified cell lines. Int J Cancer. 2010; 127:1–8. [PubMed: 20143388]
16. Lacroix M. Persistent use of "false" cell lines. Int J Cancer. 2008; 122:1–4. [PubMed: 17960586]
17. van Bokhoven A, Varella-Garcia M, Korch C, Hessels D, Miller GJ. Widely used prostate carcinoma cell lines share common origins. Prostate. 2001; 47:36–51. [PubMed: 11304728]
18. van Bokhoven A, Varella-Garcia M, Korch C, Johannes WU, Smith EE, Miller HL, Nordeen SK, Miller GJ, Lucia MS. Molecular characterization of human prostate carcinoma cell lines. Prostate. 2003; 57:205–25. [PubMed: 14518029]
19. van Bokhoven A, Varella-Garcia M, Korch C, Miller GJ. TSU-Pr1 and JCA-1 cells are derivatives of T24 bladder carcinoma cells and are not of prostatic origin. Cancer Res. 2001; 61:6340–4. [PubMed: 11522622]
20. Dirks WG, Drexler HG. Online verification of human cell line identity by STR DNA typing. Methods Mol Biol. 2011; 731:45–55. [PubMed: 21516397]

21. Dirks WG, Faehnrich S, Estella IA, Drexler HG. Short tandem repeat DNA typing provides an international reference standard for authentication of human cell lines. ALTEX. 2005; 22:103–9. [PubMed: 15953965]
22. Masters JR, Thomson JA, Daly-Burns B, Reid YA, Dirks WG, Packer P, Toji LH, Ohno T, Tanabe H, Arlett CF, Kelland LR, Harrison M, Virmani A, Ward TH, Ayres KL, Debenham PG. Short tandem repeat profiling provides an international reference standard for human cell lines. Proc Natl Acad Sci U S A. 2001; 98:8012–7. [PubMed: 11416159]
23. Parson W, Kirchebner R, Muhlmann R, Renner K, Kofler A, Schmidt S, Kofler R. Cancer cell line identification by short tandem repeat profiling: power and limitations. FASEB J. 2005; 19:434–6. [PubMed: 15637111]
24. Cell line misidentification: the beginning of the end. Nat Rev Cancer. 2010; 10:441–8. [PubMed: 20448633]
25. Lorenzi PL, Reinhold WC, Varma S, Hutchinson AA, Pommier Y, Chanock SJ, Weinstein JN. DNA fingerprinting of the NCI-60 cell line panel. Mol Cancer Ther. 2009; 8:713–24. [PubMed: 19372543]
26. Mouchawar J, Korch C, Byers T, Pitts TM, Li E, McCredie MR, Giles GG, Hopper JL, Southey MC. Population-based estimate of the contribution of TP53 mutations to subgroups of early-onset breast cancer: Australian Breast Cancer Family Study. Cancer Res. 2010; 70:4795–800. [PubMed: 20501846]
27. Gologan A, Sepulveda AR. Microsatellite instability and DNA mismatch repair deficiency testing in hereditary and sporadic gastrointestinal cancers. Clin Lab Med. 2005; 25:179–96. [PubMed: 15749237]
28. Hecht JL, Mutter GL. Molecular and pathologic aspects of endometrial carcinogenesis. J Clin Oncol. 2006; 24:4783–91. [PubMed: 17028294]
29. Lax SF. Molecular genetic pathways in various types of endometrial carcinoma: from a phenotypical to a molecular-based classification. Virchows Arch. 2004; 444:213–23. [PubMed: 14747944]
30. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. Lancet. 2005; 366:491–505. [PubMed: 16084259]
31. Albitar L, Pickett G, Morgan M, Davies S, Leslie KK. Models representing type I and type II human endometrial cancers: Ishikawa H and Hec50co cells. Gynecol Oncol. 2007; 106:52–64. [PubMed: 17490735]
32. Kuramoto H, Hamano M, Imai M. HEC-1 cells. Hum Cell. 2002; 15:81–95. [PubMed: 12227503]
33. Mo B, Vendrov AE, Palomino WA, DuPont BR, Apparao KB, Lessey BA. ECC-1 cells: a well-differentiated steroid-responsive endometrial cell line with characteristics of luminal epithelium. Biol Reprod. 2006; 75:387–94. [PubMed: 16707768]
34. Richardson GS, Dickersin GR, Atkins L, MacLaughlin DT, Raam S, Merk LP, Bradley FM. KLE: a cell line with defective estrogen receptor derived from undifferentiated endometrial cancer. Gynecol Oncol. 1984; 17:213–30. [PubMed: 6706226]
35. Way DL, Grosso DS, Davis JR, Surwit EA, Christian CD. Characterization of a new human endometrial carcinoma (RL95-2) established in tissue culture. In Vitro. 1983; 19:147–58. [PubMed: 6339371]
36. Kuramoto H, Tamura S, Notake Y. Establishment of a cell line of human endometrial adenocarcinoma in vitro. Am J Obstet Gynecol. 1972; 114:1012–9. [PubMed: 4673779]
37. Kuramoto H. Studies of the growth and cytogenetic properties of human endometrial adenocarcinoma in culture and its development into an established line. Acta Obstet Gynaecol Jpn. 1972; 19:47–58. [PubMed: 4678779]
38. Kuramoto H, Nishida M, Morisawa T, Hamano M, Hata H, Kato Y, Ohno E, Iida T. Establishment and characterization of human endometrial cancer cell lines. Ann N Y Acad Sci. 1991; 622:402–21. [PubMed: 2064198]
39. Satyaswaroop PG, Fleming H, Bressler RS, Gurpide E. Human endometrial cancer cell cultures for hormonal studies. Cancer Res. 1978; 38:4367–75. [PubMed: 698977]

40. Kuramoto H, Suzuki K. Effects of progesterone on the growth kinetics and the morphology of a human endometrial cancer cell-line. Acta Obstet Gynaecol Jpn. 1976; 23:123–32. [PubMed: 1026038]
41. Dawe CJ, Banfield WG, Morgan WD, Slatick MS, Curth HO. Growth in Continuous Culture, and in Hamsters, of Cells from a Neoplasm Associated with Acanthosis Nigricans. J Natl Cancer Inst. 1964; 33:441–56. [PubMed: 14207855]
42. Satyaswaroop, PG. Endometrial Cancer. In: Masters, JRW.; Palsson, B., editors. Human Cell Culture. Kluwer Academic Publishers; 1999. p. 71-78.
43. Nishida M, Kasahara K, Kaneko M, Iwasaki H, Hayashi K. Establishment of a new human endometrial adenocarcinoma cell line, Ishikawa cells, containing estrogen and progesterone receptors. Nihon Sanka Fujinka Gakkai Zasshi. 1985; 37:1103–11. [PubMed: 4031568]
44. Nishida M, Kasahara K, Oki A, Satoh T, Arai Y, Kubo T. Establishment of eighteen clones of Ishikawa cells. Hum Cell. 1996; 9:109–16. [PubMed: 9183638]
45. Nishida M. The Ishikawa cells from birth to the present. Hum Cell. 2002; 15:104–17. [PubMed: 12703541]
46. Lewis KA, Mullany S, Thomas B, Chien J, Loewen R, Shridhar V, Cliby WA. Heterozygous ATR mutations in mismatch repair-deficient cancer cells have functional significance. Cancer Res. 2005; 65:7091–5. [PubMed: 16103057]
47. Inoue M. Current molecular aspects of the carcinogenesis of the uterine endometrium. Int J Gynecol Cancer. 2001; 11:339–48. [PubMed: 11737463]
48. Risinger JI, Berchuck A, Kohler MF, Watson P, Lynch HT, Boyd J. Genetic instability of microsatellites in endometrial carcinoma. Cancer Res. 1993; 53:5100–3. [PubMed: 8221644]
49. Umar A, Boyer JC, Thomas DC, Nguyen DC, Risinger JI, Boyd J, Ionov Y, Perucho M, Kunkel TA. Defective mismatch repair in extracts of colorectal and endometrial cancer cell lines exhibiting microsatellite instability. J Biol Chem. 1994; 269:14367–70. [PubMed: 8182040]
50. Ignar-Trowbridge DM, Teng CT, Ross KA, Parker MG, Korach KS, McLachlan JA. Peptide growth factors elicit estrogen receptor-dependent transcriptional activation of an estrogen-responsive element. Mol Endocrinol. 1993; 7:992–8. [PubMed: 8232319]
51. Collins F, MacPherson S, Brown P, Bombail V, Williams AR, Anderson RA, Jabbour HN, Saunders PT. Expression of oestrogen receptors, ERalpha, ERbeta, and ERbeta variants, in endometrial cancers and evidence that prostaglandin F may play a role in regulating expression of ERalpha. BMC Cancer. 2009; 9:330. [PubMed: 19758455]
52. Wenger SL, Senft JR, Sargent LM, Bamezai R, Bairwa N, Grant SG. Comparison of established cell lines at different passages by karyotype and comparative genomic hybridization. Biosci Rep. 2004; 24:631–9. [PubMed: 16158200]
53. Clarke CL, Feil PD, Satyaswaroop PG. Progesterone receptor regulation by 17 beta-estradiol in human endometrial carcinoma grown in nude mice. Endocrinology. 1987; 121:1642–8. [PubMed: 3665838]
54. Satyaswaroop, PG.; Sivarajah, A.; Zaino, RJ. Hormonal control of growth of human endometrial carcinoma in nude mouse model. In: Brescani, F.; King, RG.; Lippman, ME., editors. Progress in cancer research and therapy. New York: Raven Press; 1988. p. 430-455.
55. Satyaswaroop PG, Tabibzadeh SS. Extracellular matrix and the patterns of differentiation of human endometrial carcinomas in vitro and in vivo. Cancer Res. 1991; 51:5661–6. [PubMed: 1913685]
56. Patel RR, Sengupta S, Kim HR, Klein-Szanto AJ, Pyle JR, Zhu F, Li T, Ross EA, Oseni S, Fargnoli J, Jordan VC. Experimental treatment of oestrogen receptor (ER) positive breast cancer with tamoxifen and brivanib alaninate, a VEGFR-2/FGFR-1 kinase inhibitor: a potential clinical application of angiogenesis inhibitors. Eur J Cancer. 2010; 46:1537–53. [PubMed: 20303261]
57. Yaginuma Y, Westphal H. Analysis of the p53 gene in human uterine carcinoma cell lines. Cancer Res. 1991; 51:6506–9. [PubMed: 1660340]
58. Desai NN, Kennard EA, Kniss DA, Friedman CI. Novel human endometrial cell line promotes blastocyst development. Fertil Steril. 1994; 61:760–6. [PubMed: 7512055]
59. Hombach-Klonisch S, Kehlen A, Fowler PA, Huppertz B, Jugert JF, Bischoff G, Schluter E, Buchmann J, Klonisch T. Regulation of functional steroid receptors and ligand-induced responses

in telomerase-immortalized human endometrial epithelial cells. J Mol Endocrinol. 2005; 34:517–34. [PubMed: 15821114]

60. Kniss DA, Zimmerman PD, Garver CL, Fertel RH. Interleukin-1 receptor antagonist blocks interleukin-1-induced expression of cyclooxygenase-2 in endometrium. Am J Obstet Gynecol. 1997; 177:559–67. [PubMed: 9322624]
61. Hayflick L. The establishment of a line (WISH) of human amnion cells in continuous cultivation. Exp Cell Res. 1961; 23:14–20. [PubMed: 13712490]
62. Kniss DA, Xie Y, Li Y, Kumar S, Linton EA, Cohen P, Fan-Havard P, Redman CW, Sargent IL. ED(27) trophoblast-like cells isolated from first-trimester chorionic villi are genetically identical to HeLa cells yet exhibit a distinct phenotype. Placenta. 2002; 23:32–43. [PubMed: 11869090]
63. Masters JR. False cell lines: The problem and a solution. Cytotechnology. 2002; 39:69–74. [PubMed: 19003294]
64. Geisinger KR, Kute TE, Pettenati MJ, Welander CE, Dennard Y, Collins LA, Berens ME. Characterization of a human ovarian carcinoma cell line with estrogen and progesterone receptors. Cancer. 1989; 63:280–8. [PubMed: 2910432]
65. DiSaia PJ, Sinkovics JG, Rutledge FN, Smith JP. Cell-mediated immunity to human malignant cells. A brief review and further studies with two gynecologic tumors. Am J Obstet Gynecol. 1972; 114:979–89. [PubMed: 4118903]
66. Orth K, Hung J, Gazdar A, Bowcock A, Mathis JM, Sambrook J. Genetic instability in human ovarian cancer cell lines. Proc Natl Acad Sci U S A. 1994; 91:9495–9. [PubMed: 7937795]
67. Woods LK, Morgan RT, Quinn LA, Moore GE, Semple TU, Stedman KE. Comparison of four new cell lines from patients with adenocarcinoma of the ovary. Cancer Res. 1979; 39:4449–59. [PubMed: 498076]
68. Bast RC Jr, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest. 1981; 68:1331–7. [PubMed: 7028788]
69. Berkowitz R, Kabawat S, Lazarus H, Colvin R, Knapp R, Bast RC Jr. Comparison of a rabbit heteroantiserum and a murine monoclonal antibody raised against a human epithelial ovarian carcinoma cell line. Am J Obstet Gynecol. 1983; 146:607–12. [PubMed: 6346879]
70. Langdon SP, Lawrie SS, Hay FG, Hawkes MM, McDonald A, Hayward IP, Schol DJ, Hilgers J, Leonard RC, Smyth JF. Characterization and properties of nine human ovarian adenocarcinoma cell lines. Cancer Res. 1988; 48:6166–72. [PubMed: 3167863]
71. Buick RN, Pullano R, Trent JM. Comparative properties of five human ovarian adenocarcinoma cell lines. Cancer Res. 1985; 45:3668–76. [PubMed: 4016745]
72. Hills CA, Kelland LR, Abel G, Siracky J, Wilson AP, Harrap KR. Biological properties of ten human ovarian carcinoma cell lines: calibration in vitro against four platinum complexes. Br J Cancer. 1989; 59:527–34. [PubMed: 2653399]
73. Watanabe Y, Koi M, Hemmi H, Hoshai H, Noda K. A change in microsatellite instability caused by cisplatin-based chemotherapy of ovarian cancer. Br J Cancer. 2001; 85:1064–9. [PubMed: 11592780]
74. Loh SY, Mistry P, Kelland LR, Abel G, Harrap KR. Reduced drug accumulation as a major mechanism of acquired resistance to cisplatin in a human ovarian carcinoma cell line: circumvention studies using novel platinum (II) and (IV) ammine/amine complexes. Br J Cancer. 1992; 66:1109–15. [PubMed: 1457352]
75. Nio Y, Zighelboim J, Berek JS, Bonavida B. Sensitivity of fresh and cultured ovarian tumor cells to tumor necrosis factor, interferon-alpha 2, and OK-432. Cancer Immunol Immunother. 1988; 27:246–54. [PubMed: 3180149]
76. Andrews PA, Murphy MP, Howell SB. Differential potentiation of alkylating and platinating agent cytotoxicity in human ovarian carcinoma cells by glutathione depletion. Cancer Res. 1985; 45:6250–3. [PubMed: 4063975]
77. Eva A, Robbins KC, Andersen PR, Srinivasan A, Tronick SR, Reddy EP, Ellmore NW, Galen AT, Lautenberger JA, Papas TS, Westin EH, Wong-Staal F, Gallo RC, Aaronson SA. Cellular genes analogous to retroviral onc genes are transcribed in human tumour cells. Nature. 1982; 295:116–9. [PubMed: 6173755]

78. Ozols RF, Hogan WM, Young RC. Direct cloning of human ovarian cancer in soft agar: clinical limitations and pharmacologic applications. Recent Results Cancer Res. 1984; 94:41–50. [PubMed: 6494588]

79. Yuan Y, Kim WH, Han HS, Lee JH, Park HS, Chung JK, Kang SB, Park JG. Establishment and characterization of human ovarian carcinoma cell lines. Gynecol Oncol. 1997; 66:378–87. [PubMed: 9299249]

80. Berchuck A, Rodriguez G, Olt G, Whitaker R, Boente MP, Arrick BA, Clarke-Pearson DL, Bast RC Jr. Regulation of growth of normal ovarian epithelial cells and ovarian cancer cell lines by transforming growth factor-beta. Am J Obstet Gynecol. 1992; 166:676–84. [PubMed: 1536252]

81. Young TN, Rodriguez GC, Moser TL, Bast RC Jr, Pizzo SV, Stack MS. Coordinate expression of urinary-type plasminogen activator and its receptor accompanies malignant transformation of the ovarian surface epithelium. Am J Obstet Gynecol. 1994; 170:1285–96. [PubMed: 8178854]

82. King AE, Fleming DC, Critchley HO, Kelly RW. Regulation of natural antibiotic expression by inflammatory mediators and mimics of infection in human endometrial epithelial cells. Mol Hum Reprod. 2002; 8:341–9. [PubMed: 11912282]

83. Banerjee P, Sapru K, Strakova Z, Fazleabas AT. Chorionic gonadotropin regulates prostaglandin E synthase via a phosphatidylinositol 3-kinase-extracellular regulatory kinase pathway in a human endometrial epithelial cell line: implications for endometrial responses for embryo implantation. Endocrinology. 2009; 150:4326–37. [PubMed: 19556419]

84. Wang P, Julian JA, Carson DD. The MUC1 HMFG1 glycoform is a precursor to the 214D4 glycoform in the human uterine epithelial cell line, HES. Biol Reprod. 2008; 78:290–8. [PubMed: 17989354]

85. Menkhorst E, Salamonsen L, Robb L, Dimitriadis E. IL11 antagonist inhibits uterine stromal differentiation, causing pregnancy failure in mice. Biol Reprod. 2009; 80:920–7. [PubMed: 19144959]

86. Bukulmez O, Hardy DB, Carr BR, Auchus RJ, Toloubeydokhti T, Word RA, Mendelson CR. Androstenedione up-regulation of endometrial aromatase expression via local conversion to estrogen: potential relevance to the pathogenesis of endometriosis. J Clin Endocrinol Metab. 2008; 93:3471–7. [PubMed: 18559914]

87. Cho JH, Lee JG, Yang YI, Kim JH, Ahn JH, Baek NI, Lee KT, Choi JH. Eupatilin, a dietary flavonoid, induces G2/M cell cycle arrest in human endometrial cancer cells. Food Chem Toxicol. 2011; 49:1737–44. [PubMed: 21554918]

88. Kim JH, Yang YI, Lee KT, Park HJ, Choi JH. Costunolide induces apoptosis in human endometriotic cells through inhibition of the prosurvival Akt and nuclear factor kappa B signaling pathway. Biol Pharm Bull. 2011; 34:580–5. [PubMed: 21467650]

89. Khorram O, Han G, Magee T. Cigarette smoke inhibits endometrial epithelial cell proliferation through a nitric oxide-mediated pathway. Fertil Steril. 2010; 93:257–63. [PubMed: 19022425]

90. Carino C, Olawaiye AB, Cherfils S, Serikawa T, Lynch MP, Rueda BR, Gonzalez RR. Leptin regulation of proangiogenic molecules in benign and cancerous endometrial cells. Int J Cancer. 2008; 123:2782–90. [PubMed: 18798554]

91. Jabbour HN, Boddy SC. Prostaglandin E2 induces proliferation of glandular epithelial cells of the human endometrium via extracellular regulated kinase 1/2-mediated pathway. J Clin Endocrinol Metab. 2003; 88:4481–7. [PubMed: 12970327]

92. Luo X, Xu J, Chegini N. Gonadotropin releasing hormone analogue (GnRHa) alters the expression and activation of Smad in human endometrial epithelial and stromal cells. Reprod Biol Endocrinol. 2003; 1:125. [PubMed: 14678567]

93. Luo X, Xu J, Chegini N. The expression of Smads in human endometrium and regulation and induction in endometrial epithelial and stromal cells by transforming growth factor-beta. J Clin Endocrinol Metab. 2003; 88:4967–76. [PubMed: 14557482]

94. Aboussahoud W, Aflatoonian R, Bruce C, Elliott S, Ward J, Newton S, Hombach-Klonisch S, Klonisch T, Fazeli A. Expression and function of Toll-like receptors in human endometrial epithelial cell lines. J Reprod Immunol. 2010; 84:41–51. [PubMed: 19959241]

95. Willing C, Peich M, Danescu A, Kehlen A, Fowler PA, Hombach-Klonisch S. Estrogen-independent actions of environmentally relevant AhR-agonists in human endometrial epithelial cells. Mol Hum Reprod. 2011; 17:115–26. [PubMed: 20876610]

96. Chambers AF. MDA-MB-435 and M14 cell lines: identical but not M14 melanoma? Cancer Res. 2009; 69:5292–3. [PubMed: 19549886]

97. O'Brien SJ. Cell culture forensics. Proc Natl Acad Sci U S A. 2001; 98:7656–8. [PubMed: 11438719]

98. Barallon R, Bauer SR, Butler J, Capes-Davis A, Dirks WG, Elmore E, Furtado M, Kline MC, Kohara A, Los GV, MacLeod RA, Masters JR, Nardone M, Nardone RM, Nims RW, Price PJ, Reid YA, Shewale J, Sykes G, Steuer AF, Storts DR, Thomson J, Taraporewala Z, Alston-Roberts C, Kerrigan L. Recommendation of short tandem repeat profiling for authenticating human cell lines, stem cells, and tissues. In Vitro Cell Dev Biol Anim. 2010; 46:727–32. [PubMed: 20614197]

99. Katsnelson A. Biologists tackle cells' identity crisis. Nature. 2010; 465:537. [PubMed: 20520682]

Highlights

- STR DNA profiling was used to analyze ovarian and endometrial cell lines.
- Results demonstrate significant misidentification, duplication and cross contamination.
- ECC-1 cells identified as Ishikawa cells and are not derived from EnCa-101 tumor.
- ‘Normal’ endometrial HES and hTERT-EEC cells identified as HeLa and MCF-7, respectively.
- Expanded reference DNA profiles at 16 loci for endometrial and ovarian cancer cell lines.

Table 1

Summary of STR profiles of Ishikawa and ECC-1 endometrial cancer cells and EnCa-101 tumor.

Cell Line	Amelogenin	CSF1PO	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA
Ishikawa Original	X	11, 12	18, 20	17, 18	10, 11	8.3, 11	12, 16	9, 12	9	14, (19) 20, 21 polymorphic	12.2, 14	28	21	9, 10	8	14, 18
Ishikawa '3-H-4'	X	11, 12	19, 20	16, 17	10, 11	9, 10	12, 16	9, 12	9	13, 21, 22	12.2, 14	28	21, 22	9	8	14, 17
Ishikawa 3-H-12	X	11,12, (13)	19, 20	16, 17	10, 11	9, 10	12, 13, 16	9, 12, 13	9	12, 19, 20	13.2, 14	28	20, 21	9, 10	8	14, 17
Ishikawa 3-H-12	X	11, 12	20	16, 17, (18)	10,11, (12)	9, 10	12, (13), 16	9, 12	9, (10)	13, 20	12.2,14, (15)	28	21	9,10 or 11	8	14,17 or 18
Ishikawa ER -ve	X	11, 13	20	16, 17	10, 11 10,	9, 10	12, 13, 16	9, 12	8, 9	13, 19	12.2, 14	28	20	9,10	8	14, 17
Ishikawa, ECACC, this report	X	11,12, (13)	20	15, 17	11, 12	9, 10	12, 16	9, 12, 13	9	13, 19, (14, 20)	12.2, 14	28	21	9, 10	8	14, 17
Ishikawa, ECACC	X	11,12	NT	NT	10, 11	9, 10	NT	9, 12	9	NT	NT	NT	NT	9, 10	8	14, 17
ECC-1	X	11, 12	20	16, 17	10, 11	9, 10	13, 16	9, 12,	9	12, 19	12.2,14 or 15	28	21	9, 10	8	14, 17
ECC-1 ATCC CRL- 2923	X	11, 12	NT	NT	10, 11	9, 10	NT	9, 12	9	NT	NT	NT	NT	9, 10	8	14, 17
EnCa- 101	X	13, 14	23, 27	15, 21	14, 15	11.3, 12	18, 21	10, 13	12, 13	16	13.2,14 or 15	27, 30	21	9, 9.3	8	18, 23

Number of STRs at each of 16 surveyed loci. Numbers after decimal point indicate number of bases in an incomplete STR. Commas separate allele calls for multiple peaks. Alleles in parenthesis indicate low amplitude peaks suggesting only a minor fraction of the cells in the population carry that allele. ECACC: DNA profile from European Collection of Cell Cultures; ATCC: DNA profile from American Type Culture Collection. NT: locus not tested.

Table 2

Summary of TP53 mutations and Single Nucleotide polymorphisms (SNPs).

TP53 Reference Sequence	Ishikawa Original	Ishikawa '3-H-4'	Ishikawa 3-H-12	Ishikawa 3-H-12	ECC-1	EnCa-101 Tumor
Intron 1: VNTR A_4T repeats	Homozygous 8 repeats	Homozygous 8 repeats	Homozygous 8 repeats	Homozygous 8 repeats	Homozygous 8 repeats	Heterozygous 7 & 9 repeats
Exon 4: G12069 Asp 49	Heterozygous G12069S Asp49His	Heterozygous G12069S Asp49His	Heterozygous G12069S Asp49His	Heterozygous G12069S Asp49His	Heterozygous G12069S Asp49His	
Intron 4: G12299			Heterozygous G12299K			
Intron 4: Poly A_7 12718-12724	Heterozygous 12724insA Poly A_7 / A_8					
Intron 5: G12786						Homozygous G12786T SNP
Intron 5: C13253						Heterozygous C13253Y SNP
Intron 6: G13642						Heterozygous G13462K SNP
Intron 6: Poly A_9 13756-13764	Heterozygous 13764delA Poly A_9 / A_8					
Exon 7: A14063 Met246	Heterozygous A14063R Met246Val	Heterozygous A14063R Met246Val	Heterozygous A14063R Met246Val	Heterozygous A14063R Met246Val	Heterozygous A14063R Met246Val	
Intron 10: Poly T_7 17816-17822	Heterozygous 17822delT Poly T_7 / T_6	Heterozygous 17822delT Poly T_7 / T_6	Heterozygous 17822delT Poly T_7 / T_6	Heterozygous 17822delT Poly T_7 / T_6	Heterozygous 17822delT Poly T_7 / T_6	Homozygous/ Hemizygous Poly T_7

Tumor protein p53 (TP53) genomic DNA, from multiple independent samples of each cell line, was sequenced as described in Material and Methods. The normal reference normal is GenBank HSP53G, a.k.a. X54156, which is used by the International Agency for Research on Cancer IARC (http://www-p53.iarc.fr). A blank cell in the table indicates the DNA sequence matches the reference / normal sequence. VNTR: Variable Number Tandem Repeat. Symbols- K: G & T; R: A & G; S:G & C; Y:C & T; del: nucleotide deletion; ins: nucleotide insertion.

Table 3

Summary of STR profiles of normal immortalized endometrial epithelial cells.

Cell Line	Amelogenin	CSF1PO	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA
hTERT -EEC- B37	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
hTERT -EEC- 15	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
hTERT -EEC- 17	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	25	6	12	15
hTERT -EEC- 38	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
hTERT -EEC- 49	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
MCF-7 (HTB-22) this report	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
MCF-7 NCI-60	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23, 25	6	9, 12	14, 15
MCF-7ATCC (HTB- 22)	X	10	NT	NT	11, 12	8, 9	NT	11	11, 12	NT	NT	NT	NT	6	9, 12	14, 15
HES	X	9, 10	17	15, 18	11, 12	8, 12	12, 13	13.3	9, 10	16	13, 14	27, 28	21	7	8, 12	16, 18
HeLa this report	X	9, 10	17	15, 18	11, 12	8, 12	12, 13	12, 13.3	9, 10	16	13, 14	27, 28	18, 21	7	8, 12	16, 18
HeLa ATCC (CCL- 2)	X	9, 10	NT	NT	11, 12	8, 12	NT	12, 13.3	9, 10	NT	NT	NT	NT	7	8, 12	16, 18
HeLaS3ATCC (CCL- 2.2)	X	9, 10	NT	NT	11, 12	8, 12	NT	13.3	9, 10	NT	NT	NT	NT	7	8, 12	16, 18
WISH ATCC (CCL- 25)	X	9, 10	NT	NT	11, 12	8, 12	NT	13.3	9, 10	NT	NT	NT	NT	7	8, 12	16, 18

Number of STRs at each of 16 surveyed loci. Numbers after decimal point indicate number of bases in an incomplete STR. Commas separate allele calls for multiple peaks. NT: not tested. Numbers following hTERT-ECC indicate clones. Samples were analyzed in duplicate independent reactions. MCF-7 breast cancer cells reference STR profiles from ATCC (HTB-22) and NCI-60 panel [25]. HeLa and WISH reference profiles from ATCC database.

Table 4

STR profiles of cervical and other cancer cell lines misclassified as ovarian.

Cell Line	Amelogenin	CSF1PO	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S3 17	D16S539	D18S51	D19S433	D21S11	FGA	THO1	TPOX	vWA	HPV
A2008	X	11	18	16	12	9, 10	14	11, 12	12, 13	12	13, 15.2	30, 31	23	8, 9.3	8, 10	15, 17	+
C13	X	11	18	16	12	9	14	11, 12	12, 13	12	15.2	30, 31	23	8, 9.3	8, 10	15, 17	NT
ME- 180	X	11	18	16	12	9, 10	14	11, 12	12, 13	12	13, 15.2	30, 31	23	8, 9.3	8, 10	15, 17	NT
OV2008	X	11	18	16	12	9, 10	14	11, 12	12, 13	12	13, 15.2	30, 31	23	8, 9.3	8, 10	15, 17	+
ME- 180ATCC (HTB- 33)	*X*	***11***	***NT***	***NT***	***12***	***9, 10***	***NT***	***11, 13***	***12, 13***	***NT***	***NT***	***NT***	***NT***	***8, 9.3***	***8, 10***	***15, 17***	*+*
SKOV 4	X	12	23, 25	16	11, 12	10	10, 14	13	13, 14	15, (17), 18	11, 13	29, 31, 32	21, 26	7, 8	9	18,20 (19)	NT
SKOV 6	X	12	23, 25	16	11, 12	10	10, 14	13	13, 14	15, (17), 18	11, 13	29,30, 31, 32	21, 26	7, 8	9	18, 20	NT
C-33A	X	12	23, 25	16	11, 12	10	10, 14	13	13, 14	15, (17), 18	11, 13, 14	29, 30, 31	21, 26	7, 8	9	18, 20	NT
C-33A ATCC (HTB- 31)	*X*	***12***	***NT***	***NT***	***11, 12***	***10***	***NT***	***13***	***13, 14***	***NT***	***NT***	***NT***	***NT***	***7, 8***	***9***	***18, 20***	*−*
BG-1	X	10	21, 23	16	11, 12	8, 9	10, 14	11	11, 12	14	13, 14	30	23,24, 25	6	9, 12	14, 15	NT
MCF-7NCI- 60	*X*	***10***	***21, 23***	***16***	***11, 12***	***8, 9***	***10, 14***	***11***	***11, 12***	***14***	***13, 14***	***30***	***23, 25***	***6***	***9, 12***	***14, 15***	***NT***
MCF-7ATCC (HTB- 22)	*X*	***10***	***NT***	***NT***	***11, 12***	***8, 9***	***NT***	***11***	***11, 12***	***NT***	***NT***	***NT***	***NT***	***6***	***9, 12***	***14, 15***	***NT***
CH1	X	9, 12, 13	24	15	11	9	14, 15	9, 10	9, 12	15, 18	13	29, 31.2	24	7, 9	11	15, 17	NT
CH1- cisR	X	9, 13	24	15	11	9	14, 15	9, 10	9, 12	15, 18	13	29, 31.2	24	7, 9	11	15, 17	NT
222	X	9, 13	24	15	11	9	14, 15	9, 10	9, 12	15, 18	13	29, 31.2	24	7, 9	11	15, 17	NT
PA-1JCRB(9061)	*X*	***9, 12***	***NT***	***NT***	***11***	***9***	***NT***	***9, 10***	***9, 12***	***NT***	***NT***	***NT***	***NT***	***7, 9***	***11***	***15, 17***	***NT***
NOSE 06	X	8, 10	20, 24	14, 16	11	10	14	11	10, 13	12, 16	13, 14	32.2, 33.2	21, 24	6, 9.3	6, 8	19	NT
NOSE 07	X	8, 10	20, 24	14, 16	11	10	14	11	10, 13	12, 16	13, 14	32.2, 33.2	21, 24	6, 9.3	6, 8	19	NT
DOV- 13	X	8, 10	20, 24	14, 16	11	10	14	11	10, 13	12, 16	13, 14	32.2, 33.2	21, 24	6, 9.3	6, 8	19	NT

Number of STRs at each of 16 surveyed loci. Numbers after decimal point indicate number of bases in an incomplete STR. Commas separate allele calls for multiple peaks. amplitude peaks suggesting only a minor fraction of the cells in the population carry that allele. NT: allele not tested. ATCC are reference DNA profiles from the American Type papilloma virus status (+: positive; -: negative). MCF-7 breast cancer cells reference STR profiles from ATCC (HTB-22) and NCI-60 panel.