



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

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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 was performed.

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 were genotyped as Ishikawa cells, MCF-7 breast cancer cells, 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.

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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

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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 to 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 A₄T) 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 Fig. 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 the 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-EEC cells 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 cells, the first to be derived from a human endometrial carcinoma [32,36,37], exhibited a unique profile (Table S3). HEC-1A, derived from the same patient, 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 adenocarcinoma [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 the **Materials and methods** section. 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 origin from the same patient. Identical alleles were present at several loci (CSF1PO, D5S818, D16S539, D21S11, TH01 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, while 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].

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	TH01	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	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	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	10, 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. X: only the amelogenin allele on the X chromosome was detected.

Upon STR and MSI analyses, ECC-1 samples exhibited DNA profiles essentially identical to Ishikawa 3-H-12 cells (Tables 1 and 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. and 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 that 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 A₄T, 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 cells 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 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 ₄ T repeats	Homozygous 8 repeats	Homozygous 8 repeats	Homozygous 8 repeats	Homozygous 8 repeats	Homozygous 8 repeats	Heterozygous 7 and 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 the **Materials and methods** section. 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 that matches the reference/normal sequence. VNTR: Variable Number Tandem Repeat. Symbols – K: G and T; R: A and G; S:G and C; Y:C and T; del: nucleotide deletion; ins: nucleotide insertion.

(Table 3) indicated that 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

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	23, 25	6	9, 12	14, 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-7 ATCC (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
HeLaS3 ATCC (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-EEC 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. X: only the amelogenin allele on the X chromosome was detected.

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 were 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

Table 4
STR profiles of cervical and other cancer cell lines misclassified as ovarian.

Cell line	Amelogenin	CSF1PO	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	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-180 ATCC (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	+
SKOV4	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
SKOV6	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-33 A	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-33 A 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-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	NT
MCF-7 ATCC (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-1 JCRB (9061)	X	9, 12	NT	NT	11	9	NT	9, 10	9, 12	NT	NT	NT	NT	7, 9	11	15, 17	NT
NOSE06	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
NOSE07	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. Alleles in parentheses indicate low amplitude peaks suggesting only a minor fraction of the cells in the population carry that allele. NT: allele not tested. ATCC is a reference DNA profile from the American Type Culture Collection. HPV: human papilloma virus status (+: positive; -: negative). MCF-7 breast cancer cells reference STR profiles from ATCC (HTB-22) and NCI-60 panel. X: only the amelogenin allele on the X chromosome was detected.

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 is 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/TYKnu cisR 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 IC₅₀ [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 to 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 has 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 the 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 data to this article can be found online at <http://dx.doi.org/10.1016/j.jgyno.2012.06.017>.

Conflict of interest statement

No conflict of interest.

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