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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.

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 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 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 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 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, 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, 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].