

Deoxyribonucleic Acid Profiling Analysis of 40 Human Thyroid Cancer Cell Lines Reveals Cross-Contamination Resulting in Cell Line Redundancy and Misidentification

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Context: Cell lines derived from human cancers provide critical tools to study disease mechanisms and develop novel therapies. Recent reports indicate that up to 36% of cell lines are cross-contaminated.

Objective: We evaluated 40 reported thyroid cancer-derived cell lines using short tandem repeat and single nucleotide polymorphism array analysis.

Results: Only 23 of 40 cell lines tested have unique genetic profiles. The following groups of cell lines are likely derivatives of the same cell line: BHP5-16, BHP17-10, BHP14-9, and NPA87; BHP2-7, BHP10-3, BHP7-13, and TPC1; KAT5, KAT10, KAT4, KAT7, KAT50, KAK1, ARO81-1, and MRO87-1; and K1 and K2. The unique cell lines include BCPAP, KTC1, TT2609-C02, FTC133, ML1, WRO82-1, 8505C, SW1736, Cal-62, T235, T238, Uth-104, ACT-1, HTh74, KAT18, TTA1, FRO81-2, HTh7, C643, BHT101, and KTC-2. The misidentified cell lines included the DRO90-1, which matched the melanoma-derived cell line, A-375. The ARO81-1 and its derivatives matched the HT-29 colon cancer cell line, and the NPA87 and its derivatives matched the M14/MDA-MB-4355 melanoma cell line. TTF-1 and Pax-8 mRNA levels were determined in the unique cell lines.

Conclusions: Many of these human cell lines have been widely used in the thyroid cancer field for the past 20 yr and are not only redundant, but not of thyroid origin. These results emphasize the importance of cell line integrity, and provide the short tandem repeat profiles for a panel of thyroid cancer cell lines that can be used as a reference for comparison of cell lines from other laboratories. (*J Clin Endocrinol Metab* 93: 4331–4341, 2008)

Cell lines derived from human tumors are widely used to study the mechanisms involved in cancer, as well as to serve as preclinical models to assess efficacy of novel therapies. Despite the importance of cell line integrity, cell line uniqueness or derivation from the original tumor is rarely verified, even though

repeated reports have shown that this is a long-standing problem. HeLa was the first human cell line established in 1952 and is a well-known contaminant of numerous cell lines thought to be derived from distinct tumor types (1). Additional cross-contaminations and misidentifications have been reported, and cur-

0021-972X/08/\$15.00/0

Printed in U.S.A.

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doi: 10.1210/jc.2008-1102 Received May 22, 2008. Accepted August 12, 2008.

First Published Online August 19, 2008

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Abbreviations: ATC, Anaplastic thyroid carcinoma; ATCC, American Type Culture Collection; DSMZ, German Collection of Microorganisms and Cell Cultures; ECACC, European Collection for Cell Cultures; FAM, 6-carboxyfluorescein; FTC, follicular thyroid carcinoma; LOH, loss of heterozygosity; PTC, papillary thyroid cancer; qRT-PCR, quantitative RT-PCR; SNP, single nucleotide polymorphism; STR, short tandem repeat; TAMRA, 6-carboxy-tetramethylrhodamine; WT, wild type.

rent estimates indicate that 18–36% of cell lines are cross-contaminated or misidentified, including cell lines within the National Cancer Institute-60 panel (1–4). Of note, many cases of cell line cross-contamination occur at the source and are often cross-contaminated with older, more established cancer-derived cell lines, indicating that the intended cell line was never established (1, 5–9). Therefore, it is critical to evaluate cell line integrity even if cell lines are obtained from the source. Recently, the National Institutes of Health has recognized the problem and has issued a notice that cell line authentication must accompany grant applications (10).

A comprehensive analysis of commonly used human thyroid cancer cell lines is currently lacking, despite three recent reports that have identified redundant cell lines (11–13). Meireles *et al.* (11) reported that the papillary thyroid cancer (PTC)-derived FB2 and TPC1 cell lines, which both harbor the RET/PTC1 rearrangement, are genetically identical, and van Staveren (12) and Rocha (13) *et al.* reported redundancy among the KAK-1, KAT10, and KAT4 cell lines, which all share a point mutation in E-cadherin, a rare event in thyroid cancer. We used short tandem repeat (STR) profiling and single nucleotide polymorphism (SNP) array analysis to systematically evaluate the integrity of the thyroid cancer cell lines in our laboratories (3). STR profiling was originally developed for forensic applications (14) and has been established as an international reference standard for genetically profiling cell lines (3). All major cell line repositories use STR profiling to authenticate their cell lines, thus increasing the power of this approach in the form of searchable STR profile databases. In this study we genetically analyzed 40 thyroid cancer cell lines. We found significant redundancy among the cell lines and misidentification of some cell lines that are likely not of thyroid cancer origin.

Materials and Methods

Cells and cell culture

Cell lines, as well as year of first publication, and reported mutation status are listed in Table 1, and were obtained from the primary source or the American Type Culture Collection (ATCC) with the exception of the following. BCPAP cells were kindly provided by Dr. M. Santoro (Medical School, University “Federico II” of Naples, Naples, Italy). K1 and K2 cell lines were provided by Dr. Wynford-Thomas (Cardiff University, Cardiff, UK). SW1736, C643, HTh7, and HTh74 cells were provided by Dr. K. Ain (University of Kentucky, Lexington, KY) with permission from Dr. N.-E. Heldin (University Hospital, Uppsala, Sweden), and the TPC1 cells were kindly provided by Dr. S. Jhiang (Ohio State University, Columbus, OH). Although we were unable to obtain these cell lines from the source, these cell lines matched their respective STR profiles shown in previously published studies (11, 15), and/or in the German Collection of Microorganisms and Cell Cultures (DSMZ) and European Collection for Cell Cultures (ECACC) databases at all loci tested in common (Table 2). In most of these cases, we were unable to obtain cell lines from the source due to a lack of current stocks. All cell lines were grown at 37 C with 5% CO₂ in a humidified environment (media used for individual cell lines are available upon request). To avoid cross-contamination, cell lines were cultured separately in a sterile tissue culture hood, which was UV irradiated between each cell line, and a separate bottle of media was used for each cell line.

DNA profiling protocol

Genomic DNA was prepared separately for each cell line in a clean hood using DNeasy Tissue kit (QIAGEN, Inc., Valencia, CA). To avoid cross-contamination, Pipetman were UV irradiated between each sample, and filtered pipette tips were used throughout the DNA preparation. The earliest or original passage of each cell line received in our laboratories was analyzed in each case. The Applied Biosystems Profiler Plus kit (ABI no. 4303326; Foster City, CA) was used with modifications. Genomic DNA (~1 ng) was used as template, and cycling conditions were those suggested by the manufacturer, and multiple aliquots of diluted PCRs were electrophoresed in an ABI 3730 genetic analyzer using the POP7 polymer and 50 cm. The data were collected using ABI's Data Collection software version 3.0, and the sizes of the PCR products for the different STR loci were determined using GeneMapper 4.0. The identities of the products were determined by comparing the product sizes with those of the different alleles present in the standard allelic ladder in the Profiler Plus kit. A no DNA template control was included in all stages of the STR analysis to exclude contamination. The profiles were compared visually or by using a searchable database (Korch, C. and J. West, personal communication) containing in-house data and data downloaded from the ATCC web site (www.atcc.org/common/cultures/str.cfm). The ECACC and DSMZ databases were manually searched with the STR profiles.

SNP arrays

The genetic identity of some of the commonly used thyroid cancer cell lines was further characterized by SNP genotyping, using Affymetrix 250K SNP arrays (Santa Clara, CA). Each sample was restricted with *NspI*, labeled, and hybridized to the Affymetrix GeneChip Human Mapping 250K SNP array, according to the manufacturer's instructions. Genotyping calls were generated using GType 1.1.

BRAF mutation analysis

Exon 15 of BRAF was amplified from genomic DNA (120 ng) using standard touchdown PCR methods with the following primers: BRAF-forward, 5'-TCATAATGCTTGCTCTGATAGGA-3'; and BRAF-reverse, 5'-GGCCAAAAATTTAATCAGTGGA-3'. Residual primers and deoxynucleotide triphosphates were removed before sequencing using the ExoSAP-IT kit (USB Corp., Cleveland, OH). DNA samples were sequenced using the BRAF-For primer by the University of Colorado Cancer Center DNA Sequencing & Analysis Core (<http://loki.uchsc.edu>).

RET/PTC1 analysis

Total RNA was isolated from cells/tissues using RNeasy Mini Kit (QIAGEN) and reverse transcribed using Moloney Murine leukemia virus reverse transcriptase (Life Technologies, Inc., Gaithersburg, MD). The primers used for RET/PTC1 amplification were: RET/PTC1 (F) 5'-ATTGTCATCTCGCCGTC-3' and RET/PTC1 (R) 5'-CTTTCAG-CATCTTCACGG-3'. The final PCR mixture contained 200 nM each of forward and reverse primers, 1× SYBR PCR mix (Applied Biosystems), and 5 μl cDNA template. Real-time PCR was performed with an ABI Prism 7700 sequence detector (Applied Biosystems). The thermal cycling conditions were: 2 min at 50 C, 10 min at 95 C, followed by 40 cycles of 30 sec at 95 C, 30 sec at 45 C, and 1 min at 72 C. Dissociation curves were recorded after each run, and the amplified products were visualized by 2% agarose gel electrophoresis.

Analysis of Pax-8 and TTF-1 by real-time quantitative RT-PCR (qRT-PCR)

The mRNAs for human Pax-8 and TTF-1 were measured by real-time qRT-PCR using ABI Prism 7700 Sequence detector, as previously described (16). The following primer/probe sequences were used: Pax-8 (F) 5'-GGACTACAAACGCCAGAACCC-3', Pax-8 (R) 5'-GGGCACAGTGTCAATTGTCACA-3', PAX8 (Probe) 6FAM- AC-

TABLE 1. Summary of cell lines

Cell line	Year ^a	Type ^b	Patient gender	Mutation (reported)	Refs. ^c
NPA87	1987	Papillary		BRAF(V600E) (22)	50
BHP5-16	1996	Papillary		BRAF(V600E) (51)	52
BHP17-10	1996	Papillary		BRAF(V600E) (53)	52
BHP14-9	1996	Papillary		BRAF(V600E) (53)	52
KAT5	1992	Papillary	Male	BRAF(V600E) (53)	54
KAT10	1993	Papillary	Female	BRAF(V600E) (24)	55
TPC 1	1987	Papillary		RET/PTC 1 (23)	31
BHP2-7	1997	Papillary	Female	RET/PTC 1 (51)	36
BHP10-3	1997	Papillary	Female	RET/PTC 1 (53)	36
BHP7-13	1997	Papillary	Female	RET/PTC 1 (53)	36
K1	1997	Papillary		BRAF(V600E), PI3K (E542K)	56
K2	1997	Papillary		BRAF(V600E) (57), PI3K (E542K)	56
BCPAP	1992	Papillary	Female	BRAF(V600E) (57)	58
KTC-1	2000	Papillary	Male	BRAF V600E (23)	32
KAT50	1998	Differentiated	Male		59
TT2609-CO2	1996	Follicular	Male		60
FTC 133	1989	Follicular	Male		61, 62
ML1	2000	Follicular	Female		63
WRO82-1	1982	Follicular	Female	BRAF WT (23)	64
MRO87-1	1987	Follicular		BRAF WT (37), BRAF V600E (24)	65
KAK1	1992	Follicular adenoma		BRAF V600E (24)	66
KAT7	1999	Benign follicular hyperplasia		BRAF V600E (27)	67
KAT4	1992	Anaplastic	Female	BRAF(V600E) (24, 26)	68
FRO81-2	1981	Anaplastic		BRAF(V600E) (23)	69
ARO81-1	1981	Anaplastic		BRAF(V600E) (24, 26)	70
DRO90-1	1990	Anaplastic		BRAF(V600E) (24)	71
8505C	1993	Anaplastic	Female	BRAF V600E (11)	72
SW1736		Anaplastic		BRAF(V600E) (24)	24
Cal-62	1988	Anaplastic	Female	BRAF WT (26)	73
T235	2007	Anaplastic	Female		74
T238	2007	Anaplastic	Female		74
Uth-104	1996	Anaplastic	Female	BRAF V600E (15)	15
ACT-1		Anaplastic			75
HTh7	1983	Anaplastic		BRAF WT (15)	76
HTh74	1991	Undifferentiated		BRAF WT (11)	77
KAT18		Anaplastic		BRAF WT (24)	55
TTA1		Anaplastic			78
C643	1996	Anaplastic	Male	HRAS (G13R) (11)	79
BHT101	1993	Anaplastic	Female	BRAF V600E (26)	80
KTC-2	2003	Anaplastic	Female		81
M14/MDA-MB-435	1976	Melanoma		BRAF V600E (20, 21)	19
HT-29	1964	Colon cancer	Female	BRAF V600E (29)	30
A-375	1973	Melanoma	Female	BRAF V600E (40)	46

^a Year established or first described, if known.^b Stated tumor tissue of origin.^c Earliest known reference of cell line.

CATGTTTGCCTGGGAGATCCGA-TAMRA, TTF-1 (F) 5'-AC-GACTCCGTTCTCAGTGTCTG-3', TTF-1 (R) 5'-GCCCTCCA TGCCCACTTT-3', TTF1 (probe) 6FAM- CATCTTGAGTCCCCT-GGAGGAAAGCTACA-TAMRA. The TaqMan probes were purchased from ABI, 5'-labeled with 6-carboxyfluorescein (FAM) and 3'-labeled with 6-carboxy-tetramethylrhodamine (TAMRA). Thermal cycling conditions were as follows, and reactions were performed in duplicate. Reverse transcription was performed at 48 C for 30 min, followed by activation of AmpliTaq Gold at 95 C for 10 min, followed by 40 cycles of amplification at 95 C for 15 sec and 60 C for 1 min. Each PCR run included the standard curve (10-fold serially diluted human normal thyroid RNA), test samples, no-template, and no reverse transcriptase controls. The standard curve was used to calculate the relative amounts of targets in test samples. Quantities of Pax-8 and TTF1 in test samples were normalized to the corresponding 18s rRNA

(PE ABI, P/N 4308310). Genomic DNA was isolated from normal and papillary thyroid carcinoma tissue (Cooperative Human Tissue Network, National Cancer Institute, Bethesda, MD), or anaplastic thyroid carcinoma (ATC) tissue (Mayo Clinic, Jacksonville, FL). Tissue sample no. 9N was used for the standard curve.

Results

The PTC-derived BHP5-16, BHP17-10, BHP14-9, and NPA87 cells are genetically identical to each other and the MDA-MB-435/M14 melanoma cell line

DNA profiling using STRs was performed on the 40 cell lines using 10 different genetic markers, including nine STR loci

TABLE 2. DNA profiling of cancer cell lines

Cell line	D3S1358	vWA	FGA	Amelogenin	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
<i>MDA-MB-4355^a</i>	14 ^b	16, 18 ^{b,c}	21 ^b	X ^{b,c}	13 ^b	30 ^b	13 ^b	12 ^{b,c}	12 ^{b,c}	8, 10 ^{b,c}
NPA87 ^a	14, 16	16, 18	21	X	13	30	13, 17	11, 12	12	8
BHP 5-16 ^a	14, 16	16, 18	21	X	13	30	13, 17	11, 12	12	8, 10
BHP 17-10 ^a	14, 16	16, 18	21	X	13	30	13, 17	11, 12	12	8, 10
BHP 14-9 ^a	14, 16	16, 18	21	X	13	30	13, 17	11, 12	12	8, 10
<i>HT-29^a</i>	15, 17	17, 19 ^c	20, 22	X ^c	10, 16	29, 30	13	11, 12 ^c	11, 12 ^c	10 ^c
ARO81-1,8 ^{a,d}	15, 17	17, 19	20, 22	X	10, 16	30	13	11, 12	11	10
MRO87-1 ^a	15, 17	17, 19	20, 22	X	10, 16	30	13	11, 12	11	10
KAT5 ^a	15, 17	17, 19	20, 22	X	10, 16	30	13	11, 12	11	10
KAT10 ^a	15, 17	17, 19	20, 22	X	10, 16	30	12, 13	11, 12	11	10
KAT4 ^a	15, 17 ^e	17, 19 ^e	20, 22 ^e	X ^e	10, 16 ^e	30 ^e	13 ^e	11, 12 ^e	11 ^e	10 ^e
<i>A-375^{a,f}</i>	15, 17	16, 17 ^c	20, 23	X ^c	11, 14	29, 30	12, 17	12 ^c	11, 14 ^c	9 ^c
DRO90-1 ^{a,f}	15, 17	16, 17 ^c	20, 23	X ^c	11, 14	29, 30	12, 17	12 ^c	11, 14 ^c	9 ^c
TPC1 ^a	16, 17	14, 18	20, 21	X	11, 17	30, 31.2	13, 16	8, 10	11, 12	11
BHP 2-7 ^a	16, 17	14, 18	20, 21	X	11, 17	30, 31.2	13, 16	8, 10	11, 12	11
BHP 10-3 ^a	16, 17	14, 18	20, 21	X	11, 17	30, 31.2	13, 16	8, 10	11, 12	11
BHP 7-13 ^a	16, 17	14, 18	20, 21	X	11, 17	30, 31.2	13, 16	8, 10	11, 12	11
K1 ^a	18 ^g	17, 18 ^g	21, 24 ^g	X, Y ^g	15 ^g	30, 31.2 ^g	18 ^g	10, 11	11, 14	11
K2 ^a	18	17, 18	21, 24	X, Y	15	30, 31.2	18	10, 11	11, 14	11
BCPAP ^{a,d}	16, 17	14, 17 ^h	20, 23	X ^h	12, 13	30, 31.2	13, 17	10 ^h , 11 ^h	12 ^h	10 ^h
KTC-1 ^d	14, 15	14, 17	23, 26	X, Y	11, 14	29	12, 13	11, 12	11	11
TT2609-CO2 ^d	16	17, 18 ^h	22, 25	X, Y ^h	12, 13	28, 30	14	12 ^h	11, 13 ^h	8, 10 ^h
FTC133 ^d	15 ⁱ	15, 18 ⁱ	21 ⁱ	X ⁱ	10 ⁱ	32.2 ⁱ	11, 12 ⁱ	12 ⁱ	11 ⁱ	9, 10 ⁱ
ML1 ^d	15	17, 18 ^h	22, 25	X ^h	10, 13	31.2, 33.2	18	12, 13 ^h	10 ^h	10 ^h
WRO82-1 ^a	14, 16 ⁱ	17, 18 ⁱ	20 ⁱ	X ⁱ	13, 14 ⁱ	29, 31 ⁱ	17 ⁱ	11, 12 ⁱ	13 ⁱ	11 ⁱ
8505C ^d	16, 17 ⁱ	17, 19 ^{h,i}	23 ⁱ	X ^{h,i}	10, 13 ⁱ	28, 32.2 ⁱ	16 ⁱ	10, 11 ^{h,i}	13 ^{h,i}	10 ^{h,i}
SW1736 ^{a,d}	16, 17 ^e	16, 19 ^e	22 ^e	X ^e	13, 14 ^e	29, 31 ^e	14 ^e	12, 13 ^e	11, 12 ^e	8, 11 ^e
Cal-62 ^d	16	16 ^h	19	X ^h	13	32.2	16	9, 12 ^h	12	10 ^h
T235 ^d	14	17, 18	21, 23	X	11, 14	29	13, 19	9, 12	11, 14	8, 10
T238 ^d	16	16, 17	21, 22	X	12	30	10, 17	11	12	11, 13
Uhth-104 ^d	14 ^e	14, 16 ^e	23 ^e	X ^e	12, 13 ^e	27, 31.2 ^e	14, 19 ^e	12 ^e	12 ^e	10 ^e
ACT-1 ^d	16	17, 18	23, 24	X	11, 14	30, 31	15, 16	11, 13	10, 12	10, 11
HTH74 ^{a,d}	15 ^e	17, 19 ^e	25 ^e	X ^e	13, 15, 16 ^e	32 ^e	14 ^e	11, 12 ^e	12, 13 ^e	8, 9 ^e
KAT18 ^{a,d}	15	16	20, 21	X	14, 16	30, 31	14	10, 12	11	10
TTA1 ^d	15	17	23	X	13, 15	30	15	12, 13	12	11, 12
FRO81-2 ^a	17	16	22	X, Y	13, 14	28, 30	12, 15	10, 14	9	12, 13
HTH7 ^{a,d}	14, 15 ^e	14, 18 ^e	20, 23 ^e	X ^e	12, 13 ^e	29, 30 ^e	16 ^e	11 ^e	11, 14 ^e	8, 11 ^e
C643 ^{a,d}	15 ^e	15, 17 ^e	18, 21 ^e	X, Y ^e	11, 13 ^e	28 ^e	14, 18 ^e	11, 12 ^e	8, 10 ^e	9, 12 ^e
BHT101 ^d	16, 17	18, 19 ^h	18, 24	X ^h	13, 14, 15	29, 32.2	12, 15	10, 11 ^h	12 ^h	10, 11 ^h
KTC-2 ^d	15	17, 18	23	X	12	30	16	12	8, 12	11

Rows in *italics* indicate that this cell line is likely the original, nonthyroid cell line from which the other cell lines in this group were derived from.

^a Tested in the Haugen laboratory.

^b Also tested by Rae *et al.* (18).

^c Also tested by the ATCC.

^d Tested in the Fagin laboratory.

^e Also tested by Lee *et al.* (15).

^f Tested in the Molecular Genetics Laboratory at the Mayo Clinic.

^g Also tested by the ECACC.

^h Also tested by the DSMZ.

ⁱ Also tested by van Staveren *et al.* (12).

(D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820) and amelogenin. For these loci the probability of two samples selected at random having an identical profile is less than one in 10^{10} . As shown in Table 2, the PTC cell lines BHP5-16, BHP17-10, and BHP14-9 were identical to each other at all 10 loci. The BHP5-16, BHP17-10, and BHP14-9 were also identical to the PTC-derived NPA87 cell line at nine loci. The STR profile of the NPA87 cell line from the Haugen and Fagin laboratories was identical. BHP5-16, BHP17-10, and BHP14-9 are heterozygous at the D7S820 locus (8- and 10-repeat alleles), whereas the NPA87 cells have lost the 10-repeat allele (Table 2). These results indicate that the BHP5-16, BHP17-10, and BHP14-9 cell lines are not only identical to each other, but also to the NPA87 cell line.

The ATCC database was searched with BHP5-16, BHP17-10, BHP14-9, and NPA87 profiles, and identified the MDA-MB-435S cell line as a match at all five loci tested in common (Table 2; vWA, D5S818, D13S317, D7S820, plus amelogenin). Of note, the MDA-MB-435 breast cancer cell line has recently been identified as a derivative of the M14 melanoma cell line (17, 18). Because the original M14 cell line is not available through the ATCC, we obtained and analyzed the MDA-MB-435S genomic DNA from the ATCC and found it to have an identical STR profile to the aforementioned cancer cell lines at all loci except the D18S51, D5S818, and D3S1358 loci, where the MDA-MB-435S exhibited a loss of heterozygosity (LOH) (Table 2). These profiles are consistent with the STR profile of an original stock of the MDA-MB-435 cell line from MD Anderson Cancer Center in addition to a cell stock from the Lombardi Comprehensive Cancer Center (Table 2 and Ref. 18). The M14 melanoma cell line was established in 1976 from a metastatic amelanotic lesion (19), predating the reported establishment of the NPA87 cell line and its derivatives. Because NPA87 is a commonly used thyroid cancer cell line, we further examined this using 250K SNP arrays. This showed that NPA87 is identical to BHP5-16, BHP17-10, BHP14-9, and MDA-MB-435S/M14 at 96.1% of the polymorphic sites, confirming the genetic identity of these cell lines.

The MDA-MB-435/M14 cell line has been reported to express a heterozygous BRAF V600E mutation (20, 21). In contrast, the NPA87 cells have been reported to express both a homo/hemizygous BRAF mutation (22, 23) and a heterozygous BRAF mutation (24). The NPA87 cell line and the BHP5-16, BHP17-10, and BHP14-9 cell lines in our laboratories (B.R.H. and J.A.F.) express a homo/hemizygous BRAF V600E mutation (22) (data not shown). We analyzed the BRAF V600E mutational status in the MDA-MB-435S cells from the ATCC and found that the MDA-MB-435S cell lines express a heterozygous BRAF V600E mutation (data not shown). This difference is likely due to a LOH that occurred during culturing (25).

The thyroid cancer derived KAT5, KAT10, KAT4, KAT7, KAT50, KAK1, ARO81-1, and MRO87-1 cell lines are identical to each other and to the colon cancer-derived HT-29 cell line

The PTC-derived KAT5 and KAT10 cells, the ATC-derived KAT4 and ARO81-1, and the follicular thyroid carcinoma

(FTC)-derived MRO87-1 cells were analyzed by STR, and found to be genetically similar and likely identical. The KAT10 cells differed at the D18S51 locus, which in addition to a 13-repeat allele, also retained a 12-repeat allele (Table 2). These results are consistent with recent studies, which found the KAT4, KAT10, and FTC-derived KAK1 cells to be identical by STR profiling (12, 13). In addition, the KAT4 STR profile shown here is identical to that published recently by Lee *et al.* (15), except at the D8S1179 locus there was a 16-repeat allele in addition to the 10-repeat allele that we observed here (Table 2). These differences are likely due to clonal drift, and indicate that the KAT5, KAT10, and KAT4 cells are identical. We also found that the KAT5, KAT10, and KAT4 cell lines were not only identical to each other, but also to the MRO87-1 and ARO81-1 cell lines (Table 2). The ARO81-1 cell line from the Haugen and Fagin laboratories showed identical STR profiles. By 250K SNP array analysis, we found that the KAT7 and KAK1 cell lines from the Fagin laboratory are identical to the ARO81-1 cell lines at 97.1% of the sites, and the KAT50 and ARO81-1 cell lines share identity at 97.4% of the sites, proving that they are derivatives from the same line.

By searching the ATCC database, we discovered that the STR profiles of these cell lines matched that of the HT-29 colon cancer cell line at those loci tested in common (Table 2). Therefore, we obtained the HT-29 cell line from the ATCC and found that the STR profile of the HT-29 cell line is identical to that of the ARO81-1, MRO87-1, KAT5, KAT10, and KAT4 except at the D21S11 locus, where the HT-29 cell line retained the 29-repeat allele. The HT-29 cells also differed from the KAT10 cells at the D18S51 locus as discussed previously. By SNP 250K array genotyping, ARO81-1 and HT-29 were identical at 93.1% of the sites, confirming that they are genetically identical.

Consistent with previous studies, we found that the KAT5, KAT10, and KAT4 cells express a heterozygous BRAF mutation (24, 26, 27) (data not shown). Previous studies regarding BRAF mutational status in ARO81-1 cells have shown that these cells are homo/hemizygous (24) or heterozygous (23, 26, 27). The ARO81-1 cells in our laboratories (B.R.H. and J.A.F.) express a heterozygous BRAF mutation, consistent with that reported by two other laboratories (23, 27), and consistent with the ARO81-1 cell line matching the KAT5, KAT10, KAT4, KAK1, and KAT7 cell lines. The BRAF V600E mutation is also prevalent in colon cancer (28), and we found that the HT-29 colon cancer cell line from the ATCC expressed a heterozygous BRAF mutation, consistent with a previous report (29) and the genetic identity of these cell lines. The HT-29 colon cancer cell line was established in 1964 (30), before the establishment of the ARO81-1 cell line, which was established in 1981.

The ATC cell line DRO90-1 is genetically identical to the A-375 melanoma-derived cell line

The ATC-derived DRO90-1 cell line was profiled by STR analysis (Table 2). This cell line was unique among our panel of thyroid cancer cell lines; however, the DRO90-1 matched the STR profile of the A-375 melanoma cell line (ATCC database) at all five overlapping loci. Therefore, we obtained the earliest available passage of the A-375 cell line (passage 10 from 1972),

and we found that these two cell lines were identical at all 10 loci (Table 2). We further confirmed that the A-375 and DRO90-1 cell lines both express a homo/hemizygous BRAF mutation and lack TTF-1 and Pax-8 expression (data not shown). These results indicate that the DRO90-1 cell line (established in 1990) is genetically identical to the A-375 melanoma cell line by STR profiling.

The PTC-derived BHP2-7, BHP10-3, BHP7-13, and TPC1 cells are genetically identical

Table 2 shows that the BHP2-7, BHP10-3, and BHP7-13 cells are not only identical to each other by STR analysis, but also to the TPC1 cell line, which was the original cell line isolated (31). We have confirmed the presence of the RET/PTC1 rearrangement in the BHP2-7, BHP10-3, BHP7-13, and TPC1 cell lines by

SYBR green RT-PCR (Table 3 and data not shown). Expression levels of the thyroid-specific transcription factors, Pax-8 and TTF-1, were evaluated by qRT-PCR, and high levels of Pax-8 mRNA were observed by qRT-PCR (Table 3; 407 pg mRNA/ng rRNA), further supporting the TPC1 cell line as a cell line derived from thyroid cancer. TTF-1 expression was not detected by qRT-PCR (Table 3), consistent with previous studies (11).

The PTC-derived K1 and K2 cell lines are genetically identical

The PTC-derived K1 and K2 cell lines have identical STR profiles as shown in Table 2. The STR profiles obtained for the K1 and K2 cells in our study match the K1 STR profile provided by the ECACC (personal communication) for those loci tested in common (Table 2). We have confirmed the presence of the BRAF

TABLE 3. Mutational status and thyroid-specific gene expression in the unique thyroid cancer cell lines

Cell line	Tumor type	BRAF	RET/PTC1	Pax-8 ^a	TTF-1 ^a
BCPAP	PTC	V600E ^{b,c}	WT	229 ^b , 783 ^d	457 ^b , 598 ^d
KTC-1	PTC			651 ^d	274 ^d
K1	PTC	V600E/WT ^b	WT	627 ^b	0 ^b
TPC1	PTC	WT ^{b,d}	RET/PTC1	407 ^b	0 ^b
TT2609-CO2	FTC			0 ^d	687 ^d
FTC133	FTC			76 ^d	298 ^d
ML1	FTC			40 ^d	205 ^d
WRO82-1	FTC	V600E ^b		126 ^b	0 ^b
8505C	ATC	V600E/V600E ^d (82)		1 ^d	580 ^d
SW1736	ATC	V600E/WT ^b	WT	288 ^b , 288 ^d	37 ^b , 0 ^d
Cal-62	ATC	WT ^d (82)		288 ^d	0 ^d
T235	ATC			256 ^d	0 ^d
T238	ATC			230 ^d	0 ^d
Uhth-104	ATC			160 ^d	79 ^d
ACT-1	ATC	WT ^d (82)		80 ^d	114 ^d
HTh74	ATC	WT ^{b,d}	WT	0.6 ^b , 0 ^d	45 ^b , 7 ^d
KAT18	ATC	WT ^{b,d}	WT	1 ^b , 0.1 ^d	23 ^b , 0 ^d
TTA1	ATC	WT ^d (82)		3 ^d	0 ^d
FRO81-2	ATC	WT ^b		0.1 ^b	0 ^b
HTh7	ATC	WT ^b	WT	0.7 ^b	0 ^b
C643	ATC	WT ^{b,d}	WT	1 ^b , 0.3 ^d	0.03 ^b , 0 ^d
BHT101	ATC			0.5 ^d	0 ^d
KTC-2	ATC			0.9 ^d	0 ^d
2N ^e	NORMAL			1184	1798
2T ^f	PTC	WT	WT	1576	3356
3N ^e	NORMAL			1698	4314
3T ^f	PTC	WT	WT	259	1334
5N ^e	NORMAL			1780	2144
5T ^f	PTC	V600E/WT	WT	616	2607
6N ^e	NORMAL			1647	2296
6T ^f	PTC	V600E/WT	WT	988	2464
9N ^e	NORMAL			1024	1011
9T ^f	PTC	V600E/WT	WT	638	1387
DH ^g	ATC			571	940
W111 ^g	ATC			2221	4490

^a pg mRNA/ng rRNA.

^b Tested in the Haugen laboratory.

^c Homo/hemizygous.

^d Tested in the Fagin laboratory.

^e Normal thyroid tissue.

^f Papillary thyroid carcinoma tissue.

^g Anaplastic thyroid carcinoma tissue.

V600E mutation in addition to high levels of Pax-8 mRNA by qRT-PCR (Table 3; 627 pg mRNA/ng rRNA), which suggests that this cell line is likely of thyroid origin. TTF-1 mRNA was not detected, consistent with previous studies (11).

The PTC-derived cell lines, BCPAP and KTC-1 are unique

The PTC-derived BCPAP and KTC-1 cell line STR profiles are shown in Table 2. The BCPAP cell line does not match any of the cell lines tested in this study or any other cell line listed in the ATCC, DSMZ, or ECACC databases. The BCPAP profile reported here is identical to that reported by the DSMZ for those loci tested in common (Table 2) and also that reported by van Staveren *et al.* (12). Consistent with previous studies (11, 12), the BCPAP cells also express high levels of Pax-8 and TTF-1 mRNA, as determined by qRT-PCR (Table 3). We have also confirmed the presence of the BRAF V600E mutation (homo/hemizygous) (Table 3) (11). Likewise, the KTC-1 cells express high levels of Pax-8 and TTF-1, and express a heterozygous BRAF mutation, in agreement with previous studies (23, 32). The KTC-1 cell line does not match any other cell line listed in the ATCC, DSMZ, or ECACC databases.

The FTC-derived TT2609-CO2, FTC133, ML1, and WRO82-1 cell lines are unique

The STR profiles of the TT2609-CO2, FTC133, ML1, and WRO82-1 cell lines are shown in Table 2. The TT2609-CO2 and ML1 STR profiles are consistent with that reported in the DSMZ database, and the FTC133 STR profile is consistent with that of the ECACC (personal communication) (Table 2). The WRO82-1 STR profile from the Haugen laboratory is in agreement with a previously published profile (12), but not with that of the ECACC (personal communication) (data not shown). We found the TT2609-CO2, ML1, and FTC133 cells express wild-type (WT) BRAF, consistent with the follicular origin of these cells (Table 3). However, the WRO82-1 cell line from the Haugen laboratory expresses mutant BRAF V600E. The WRO82-1 cell line from the Haugen laboratory expresses moderate levels of Pax-8 (126 pg mRNA/ng rRNA) and undetectable TTF-1 (Table 3). In contrast, the TT2609-CO2, FTC133, and ML1 cell lines express high levels of TTF-1 (205–687 pg mRNA/ng rRNA), and low to undetectable levels of Pax-8 (0–76 pg mRNA/ng rRNA; Table 3).

The ATC-derived cell lines 8505C, SW1736, Cal-62, T235, T238, Uhth-104, ACT-1, HTh74, KAT18, TTA1, FRO81-2, HTh7, C643, BHT101, and KTC-2 are unique

The STR profiles of the 8505C, SW1736, Cal-62, T235, T238, Uhth-104, ACT-1, HTh74, KAT18, TTA1, FRO81-2, HTh7, C643, BHT101, and KTC-2 cell lines are shown in Table 2. These cells are unique among our panel of cell lines. The STR profiles of the 8505C, SW1736, Uhth-104, HTh74, HTh7, and C643 (Table 2) are in agreement with previously published profiles, with the exception of the SW1736 cell line, which showed a difference at the D3S1358 locus, the Uhth-104 cell line, which differed at the D18S51 locus, and the C643 cell line, which differed at the D18S51 locus (12, 15). The STR profiles of the 8505C, Cal-62, and BHT101 cell lines are also consistent with

their respective profiles in the DSMZ database. The STR profiles of the HTh7, HTh74, SW1736, and C643 cell lines, which were tested independently by the Fagin and Haugen laboratories, are in agreement with each other, with the exception of minor differences due to LOH. The Haugen and Fagin laboratory KAT18 STR profiles are identical. BRAF mutational status of these cell lines is in agreement with previous reports (if known), with exception of the FRO81-2 cell line from the Haugen laboratory, which express WT BRAF (Table 3). The SW1736, Cal-62, T235, T238, Uhth-104, and ACT-1 cell lines expressed moderate (80–160 pg mRNA/ng rRNA) to high (230–658 pg mRNA/ng rRNA) levels of Pax-8; and the 8505C, Uhth-104, and ACT-1 cell lines expressed high (580 pg mRNA/ng rRNA) to moderate (79–114 pg mRNA/ng rRNA) levels of TTF-1 (Table 3). The remaining cell lines expressed low to undetectable levels of Pax-8 and TTF-1.

Discussion

In this report we have genetically profiled 40 presumptive thyroid cancer cell lines. We have identified 23 unique cell lines (TPC1, K1, BCPAP, HTh7, HTh74, SW1736, C643, KAT18, 8505C, FRO81-2, WRO82-1, ACT-1, BHT101, Cal-62, FTC133, KTC-1, KTC-2, ML-1, T235, T238, TT2609-CO2, TTA1, and Uhth-104), which are presumably of human thyroid cancer origin. Of these cell lines, 12 (TPC1, K1, BCPAP, SW1736, WRO82-1, ACT-1, Cal62, FTC133, KTC-1, T235, T238, and Uhth-104) express high level of Pax-8 transcription factor mRNA, eight of these cell lines (BCPAP, 8505C, ACT-1, FTC133, KTC-1, ML-1, TT2609-CO2, and Uhth-104) express high level of TTF-1 transcription factor mRNA, and five of these cell lines (BCPAP, ACT-1, FTC133, KTC-1, and Uhth-104) express both transcription factors, further supporting that they are likely of human thyroid cancer origin. Although the lack of expression of these transcription factors does not exclude thyroid origin, especially for the anaplastic cell lines, high levels of Pax-8 and/or TTF-1 provide further evidence that these are thyroid cancer-derived cell lines. Of our cell lines tested, 12 (NPA87, BHP5-16, BHP17-10, and BHP14-9; ARO81-1, MRO87-1, KAT5, KAT10, KAT4, KAT7, KAT50, KAK1) are redundant and are not likely of thyroid origin. Six of our cell lines are redundant, but likely of thyroid origin (TPC1, BHP 2-7, BHP 10-3, and BHP 7-13; K1 and K2), and the DRO90-1 cell line is not likely of thyroid origin.

The expression levels of Pax-8 and TTF-1 in the unique cell lines reported here are in agreement with previous studies analyzing the expression of these genes in many of our cell lines, including the TPC1, BCPAP, and K1 cells (11). In other cases, including the C643, 8505C, HTh74, and WRO82-1 cell lines, we observed low to undetectable levels of Pax-8 and/or TTF-1, whereas previous studies reported expression of one or both of these transcription factors (11, 12). The reason for these differences is unclear but is likely due to different cell culture or RT-PCR conditions, as discussed below. Because the expression of tissue-specific genes is often reduced or lost in undifferentiated cancer, we also analyzed the expression of these genes in normal *vs.* thyroid tumor tissues using qRT-PCR. Interestingly, de-

creased levels of Pax-8 and TTF-1 mRNA are only observed in a subset of the tumor tissue samples compared with matched normal samples, indicating that these transcription factors are not necessarily down-regulated in papillary thyroid carcinoma (Table 3). Pax-8 mRNA levels in the PTC-derived cell lines are similar to that observed in the PTC and ATC tissue samples, and are lower in the FTC- and ATC-derived cell lines. In contrast, TTF-1 expression is lower in all of the cell lines, compared with normal, PTC, and ATC tissue samples (Table 3).

The results of our BRAF mutational analysis are also consistent with previous studies, with the exception of the FRO81-2 and WRO82-1 cell lines from the Haugen laboratory, which were found to express WT and mutant BRAF, respectively (Table 3). The Haugen laboratory WRO82-1 STR profile matches that previously published by van Staveren *et al.* (12), but not that of the ECACC (personal communication) or the Fagin laboratory (data not shown). This suggests that two different WRO82-1 and FRO81-2 cell lines have been distributed based on STR profiling and BRAF mutation. Both cell lines were obtained directly from the source by each laboratory (B.R.H. and J.A.F.).

For the cell lines we believe to be genetically identical, we and others have reported distinct responses which can seem confusing. For example, we have shown that the BHP5-16, BHP17-10, and BHP14-9 cell lines are more sensitive to retinoid and rexinoid treatment than the NPA87 cell line (16), even though we have shown that they are genetically identical by STR profiling. We believe that these differences in response are due to clonal drift, which can occur when cells are maintained in culture for long periods (33–35). In contrast, we and others have found that many of the sublines identified in this study, including the BHP2-7, BHP7-13, and BHP10-3 (16, 36), and the KAK1, KAT5, KAT7, KAT10, and ARO81-1 (37, 38) indeed respond as expected for identical cell lines, suggesting that long-term culture does not necessarily result in phenotypical drift of all cell lines.

Cell line cross-contamination and misidentification are longstanding problems, and redundancy among thyroid cancer cell lines has been previously reported (KAT5, KAT10, KAT4, KAK1; and FB2, TPC1) (11–13). After initial submission of this manuscript, the DSMZ (www.dsmz.de/) reported that the ONCO-DG-1 PTC-derived cell line (39) is actually a derivative of the OVCAR-3 ovarian cancer cell line. We have also confirmed that the ONCO-DG-1 and OVCAR-3 cell lines are identical by STR profiling (data not shown).

STR profiling is commonly used to determine genetic identity; however, STR results can be complicated by clonal drift that occurs in culture. Indeed, we observed minor differences in cell lines we believe to be identical. For example, the NPA87 cell line differed from the BHP5-16, BHP17-10, BHP14-9, and MDA-MB-435S cell lines at the D7S820 locus, where the BHP cell lines potentially gained a 10-repeat allele or alternatively, the NPA87 cell line lost the 10-repeat allele (Table 2). These cell lines also differed at the D3S1358, D18S51, and D5S818 loci by a loss or gain of an allele (Table 2). Previous studies evaluating the STR profile of two different stocks of the MDA-MB-435 cell line from the MD Anderson Cancer Center and the Lombardi Cancer Center Tissue Shared Resource also exhibit a small degree of clonal drift, which is comparable to our results (Table 2 and Ref. 18).

Despite these differences in STR profiles due to a loss or gain in alleles, in no case did we find differences in the number of allele repeats in the cell lines we believe to be identical. Finally, none of the differences in what we believe to be identical cell lines reduced the similarity between cell line profiles to less than the 80% cutoff proposed by Masters *et al.* (3). Furthermore, by 250K SNP array analysis, the NPA87 cell line and its derivatives matched the MDA-MB-435S/M14 cell line at 96.1% of the polymorphic sites, providing further evidence that these cell lines are identical.

For the cell lines that we believe to be identical, we found that these cell lines harbor identical BRAF mutations, with the exception of the NPA87, BHP5-16, BHP17-10, and BHP14-9 cell lines, which are homo/hemizygous (23) (data not shown), whereas the MDA-MB-435S/M14 cell line is BRAF heterozygous (20, 21) (data not shown). The precise reason for this difference is not known but could be due to LOH through loss of the WT allele or to uniparental disomy, either of which can occur during long-term culturing, as discussed previously, and has been shown for the BRAF WT allele at the T1799A site in other cell lines (40, 41). We also discovered that the Haugen laboratory MRO87-1 cell line expresses a heterozygous BRAF mutation, which is not consistent with the FTC-origin of this cell line but agrees with the genetic identity with the ARO81-1, KAT5, KAT10, KAT4, and HT-29 cell lines by STR profiling (Table 2). Previous studies have shown that the MRO87-1 cell line expresses either WT BRAF or mutant BRAF V600E (24, 37), suggesting that there are two different MRO87-1 cell lines in use. Again, this cell line was obtained directly from the laboratory that generated MRO87-1.

For the cell lines we believe to be of nonthyroid origin, previous studies have shown expression of thyroid specific genes, including Pax-8 and TTF-1 in the KAT-4, KAT-10, and KAK-1 cell lines (12, 42, 43). In addition, increased expression of thyroid-specific genes after pharmacological manipulation has been shown in some of the cell lines that we believe are of nonthyroid origin, including NPA87, DRO90-1, and ARO81-1 (37, 44, 45). In most cases expression of thyroid-specific genes was detected using PCR-based assays, and it is conceivable that trace amounts of these gene products may be expressed under certain conditions in cells that are not of thyroid origin. Alternatively, RT-PCR based methods can result in the amplification of nonspecific products. Indeed, we detected moderate levels of TTF-1 mRNA in some of the cell lines we believe to be of nonthyroid origin, including the ARO81-1 cell line, using SYBR green RT-PCR (data not shown). However, using this approach we noted variable melting temperatures between different cell lines (75–82°C, data not shown) and, therefore, used TaqMan qRT-PCR to measure more accurately TTF-1 and Pax-8 mRNA levels (Table 3). Using this approach we were able to specifically measure levels of TTF-1 and Pax-8 mRNA in our cell lines and did not detect expression of either of these transcription factors in the cell lines we believe to be of nonthyroid origin, including the ARO81-1 and NPA87 cells (data not shown). Lack of expression of thyroid-specific genes has been attributed to a dedifferentiated phenotype. Given the results of the current study, it is possible that the low expression of thyroid-specific genes is due to these cell lines being of nonthyroid origin.

Although the STR profiling approach used in this study represents an accurate and powerful approach for determining cell line identity, STR profiling does not provide any information regarding tissue of origin. Because original patient material from which most of these misidentified cell lines were derived is not available, we cannot definitely identify the tissue of origin for any of these cell lines based on STR or SNP profiling. However, given the time line of establishment and lack of Pax-8 and TTF-1 expression, we believe that the NPA87, ARO81-1, MRO87-1, and DRO90-1 cell lines and their respective sublines were established by cross-contamination at the source. The “90,” “81,” and “87” in the names DRO90-1, ARO81-1, MRO87-1, and NPA87 refer to the year the cell lines were established (Canlapan, D., University of California Los Angeles, Los Angeles, CA, personal communication). Given that the first publication of the A-375 cell line was in 1973 (46), we believe that the DRO90-1 is a subline of A-375 that resulted from cell line contamination.

Likewise, the M14 melanoma cell line was established in 1976 (19), and the MDA-MB-435 misidentification with the M14 cell line occurred before 1982 (17), both of which predate the establishment of the NPA87 cell line. Of note, the M14 cell line was also established at University of California Los Angeles (originally called UCLA-SO-M14) and was used by the originating investigators of the NPA87 cell line before the establishment of the NPA87 cell line (47). Thus, it is likely that the establishment of the NPA87 cell line is also due to cross-contamination.

The HT-29 colon cancer cell line was established in 1964 (30) and deposited into the ATCC in 1981 (48, 49). Previous studies have shown that the 1981 HT-29 stock deposited in the ATCC is genetically identical to an original HT-29 stock from 1967 (49), indicating that the ARO81-1 cell line and the MRO87-1 cell line from the Haugen laboratory were likely established by cross-contamination. The NPA87, ARO81-1, DRO90-1, and MRO87-1 cell lines and their respective sublines have been widely used as thyroid cancer models for the past 20 yr.

In conclusion, we have identified significant redundancy and misidentification among 20 of 40 thyroid cancer cell lines tested (50%). We found that Pax-8 and/or TTF-1 is expressed in the majority (15 of 23) of the unique cell lines. For cell lines that do not express either thyroid-specific gene, all are of anaplastic origin, suggesting that the loss of these genes could be due to the dedifferentiated phenotype. Based on the studies presented here, we believe the cell lines listed in Table 3 represent viable models to study thyroid cancer and at the time of this report, are unique.

Acknowledgments

We thank Agnes Viale and the Genomics Core Laboratory at Memorial Sloan-Kettering Cancer Center for the single nucleotide polymorphism-congenital GH array genotyping, and Dr. Edward Highsmith and the Molecular Genetics Laboratory at Mayo Clinic for short tandem repeat analysis of cell lines. We also thank Dr. Stuart Aaronson for providing the early passage A-375 cell line, and Dr. Wilhelm Dirks for providing the DSMZ short tandem repeat database for these studies. We thank Cathy Rowe and Helen Philpott for searching the European Collection of Cell Culture short tandem repeat database with our short tandem repeat data set.

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This work was supported by ACS MRSG-06-193-01-TBE (to J.P.K.); University of Colorado Cancer Center Sequencing Core P30 CA046934 (to C.K.); NIH-CA50706 (to J.A.F.); CA72597 (to J.A.F.); a Translational and Integrative Medicine Research Grant from Memorial Sloan-Kettering Cancer Center (to J.A.F.); the Margot Rosenberg Pulitzer Foundation (to J.A.F.); Florida Department of Health Grant 07BB-02-12297 (to J.A.C.), grant for rare cancers from Dr. Ellis and Dona Brunton (to J.A.C.); NIH P30CA15083 (Cancer Center Support Grant) (to R.C.S.), Mayo Clinic Research Committee (to R.C.S.); NCI CA100560 (to B.R.H.), Mary Rossick Kern and Jerome H Kern Endowment (to B.R.H.).

Disclosure Statement: The authors have nothing to disclose.

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