ORIGINAL ARTICLE

GENOTYPING OF 73 UM-SCC HEAD AND NECK SQUAMOUS CELL CARCINOMA CELL LINES

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Abstract: *Background.* We established multiple University of Michigan Squamous Cell Carcinoma (UM-SCC) cell lines. With time, these have been distributed to other labs all over the world. Recent scientific discussions have noted the need to confirm the origin and identity of cell lines in grant proposals and journal articles. We genotyped the UM-SCC cell lines in our collection to confirm their unique identity.

Method. Early-passage UM-SCC cell lines were genotyped and photographed.

Results. Thus far, 73 unique head and neck UM-SCC cell lines (from 65 donors, including 21 lines from 17 females) were genotyped. In 7 cases, separate cell lines were established from the same donor.

Conclusions. These results will be posted on the UM Head and Neck SPORE Tissue Core website for other investigators to confirm that the UM-SCC cells used in their laboratories have the correct features. Publications using UM-SCC cell lines should confirm the genotype. © 2009 Wiley Periodicals, Inc. *Head Neck* **32:** 417–426, 2010

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Additional Supporting Information may be found in the online version of this article.

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Head and neck squamous cell cancers (HNSCCs) account for 11,170 deaths annually in the United States and nearly 250,000 deaths annually worldwide. HNSCC cell lines developed from patients with cancers of various sites in the head and neck region[1] have been distributed to a wide array of institutions to study this disease. The squamous cell carcinoma cell lines developed at the University of Michigan (UM-SCCs) have been among the most widely used because many specific characteristics are known—such as relative radiation sensitivity,[2]

p53 mutation status,[3] karyotype,[4] antigen expression,[5,6] cisplatin sensitivity,[3] and integrin expression and activation[7–12]—that make these useful tools for other investigators. Until now these cell lines have not undergone extensive genetic fingerprinting analysis, which makes it difficult to readily confirm the identity of the individual cell lines.

Cell line identity can be derived from several different methods, including sequencing of DNA polymorphisms,[13] karyotyping,[14] and sequencing of hypervariable mitochondrial sequences,[15] and some groups have even suggested *TP53* sequencing[16] because the gene is frequently mutated in human cancers.[17,18] Unfortunately, these methods are limited by the time it takes to produce meaningful results, the expense of each protocol, and/or the value of the data. For example, *TP53* sequencing cannot be used to distinguish the identity of cell lines when the gene is wild type. Because of this, cross-contamination has become a frequent problem for researchers. For example, 45 of 252 novel cell lines (18%) collected in the German Cell Line Bank were found to have nonunique genotypes.[19] Thus, many researchers have concluded that there is a need for a rapid and standardized universal method for cell line identification.[20–23]

Despite the realization that genetic verification is a necessary component of cell line research, until recently, cell line genotyping was not reliable because some transformed tissue cultures have defective mismatch repair pathways, leading to increased microsatellite instability[24] and thus preventing reliable genotyping. Microsatellites are short tandem repeat (STR) loci that are highly polymorphic repetitive DNA sequence elements 2 to 7 nucleotides in length.[25,26] These STR loci are distributed throughout the human genome and alleles of STR loci can be differentiated by the number of repeat sequence (2–7 nucleotides long) copies located at each locus.[27] Because polymerase chain reaction (PCR)–based methods can be used to amplify STR loci, researchers have used radioactive, silver stain or fluorescence-based methods to detect STR loci length, after separation of the different alleles by electrophoresis. Many of these loci are made up of dinucleotide repeats that are susceptible to instability and polymerase slippage during PCR amplification.[28] The advent of commercially available assays based on amplifying tetranucleotide STR sequences, which have greater intrinsic stability than that of dinucleotide repeats, provides a much more reliable means of genetic identification.[28,29] As such, STR profiling has become a common reference for most commercially available cell lines.[23] Here, we present genotyping data obtained with 9 common tetranucleotide repeat sequences and the AMEL locus (which differs in length on the X and Y chromosomes) on 73 UM-SCC head and neck cell lines.

MATERIALS AND METHODS

Cell Culture. All of the UM-SCC cell lines were established from head and neck cancer patients who gave written informed consent in studies reviewed and approved by the University of Michigan Medical School Institutional Review Board. Current and early-passage human UM-SCC cell lines established at the University of Michigan[1–3,5,30] were retrieved from liquid nitrogen storage. Cell lines were grown in complete Dulbecco's modified Eagle's medium (cDMEM; Sigma Chemical Co, St. Louis, MO) containing 2 mM L-glutamine, 1% nonessential amino acids, 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA), and 10% fetal bovine serum, in a humidified atmosphere of 5% CO_2 at 37°C. All cell lines were tested for mycoplasma, using the MycoAlert Detection Kit (Cambrex, Rockland, ME). Contaminated cultures were treated with Plasmocin (InvivoGen, San Diego, CA) in accord with the manufacturer's protocol, and testing was repeated at monthly intervals.

Genomic DNA Purification. Exponentially growing (60% to 80% confluence) cells were trypsinized and washed in phosphate-buffered saline. Cell pellets were flash frozen at −80°C; resuspended in 500 μL of 0.1 M Tris (pH 8.0), 0.1 M ethylene diamine tetraacetic acid (EDTA), 0.4 M NaCl, 1% sodium dodecyl sulfate, and 0.3 mg/mL proteinase K (New England Biolabs, Ipswich, MA); and incubated overnight at 55°C. After incubation, 500 μL of phenol/chloroform (pH 6.7) was added (Fisher Scientific, Pittsburg, PA), and the dissolved cells were centrifuged for 10 minutes at 1700 × *g*. The upper phase containing the DNA was transferred to a new tube with 150 μL of 7.5 M ammonium acetate and 800 μL of 100% ethanol. The precipitated DNA was pelleted by centrifugation for 2 minutes at 1700 × *g*. DNA pellets were washed with 70% ethanol, air dried, and resuspended in high-performance liquid chromatography–grade H_2O.

Analysis of Genetic Loci. DNA samples were diluted to 0.10 ng/μL and were analyzed at the University of Michigan DNA Sequencing Core using the Profiler Plus PCR Amplification Kit (Applied Biosystems, Foster City, CA) in accord with the manufacturer's protocol. The 9 loci D3S1358, D5S818, D7S820, D8S1179, D13S317, D18S51, D21S11, FGA, and vWA, and the amelogenin locus were analyzed and compared with ladder control samples.

RESULTS

Genetic Profiling of UM-SCC Cell Lines. For each of the genotyped cell lines now represented in the UM Head and Neck SPORE cell line bank, the UM-SCC cell line number, the donor sex, the anatomic tumor site (specimen site and primary tumor location), the passage number of the genotyped cell line that was included in the SPORE tissue core freezer, and the alleles for each of the following microsatellite loci: *AMEL*, *D3S1358*, *D5S818*, *D7S820*, *D8S1179*, *D13S317*, *D18S51*, *D21S11*, *FGA*, and *vWA* are given in Table 1. The amelogenin locus on the X and Y chromosomes is used for sex identification; however, because some cell lines and even normal cells from older male donors lose the Y chromosome,[4,31–33] an AMEL-X genotype does not confirm that the donor is female. However, the presence of a Y signal was observed only in HNSCC cell lines derived from male donors. Of the 65 patient donors, 17 of 65 were females (26%).

In several cases it was possible to derive >1 cell line from the same donor.[1] In some cases, these were from different sites during the same procedure (UM-SCC-17A from the endolarynx; UM-SCC-17B from tumor extending outside the thyroid cartilage[34]; UM-SCC-22A from the primary site; UM-SCC-22B from a lymph node metastasis) or from different surgical procedures (UM-SCC-10A from the larynx at the time of laryngectomy and UM-SCC-10B from a submental lymph node metastasis 10 months later; UM-SCC-11A pretreatment biopsy; UM-SCC-11B post chemotherapy surgery; UM-SCC-14A wide local excision after excisional biopsy; UM-SCC-14B recurrence after surgery and radiation; UM-SCC-14C skin metastasis after chemotherapy; UM-SCC-74A surgical resection after chemotherapy and radiation; UM-SCC-74B second surgery for persistent cancer; UM-SCC-81A laryngeal primary; UM-SCC-81B tonsil primary).[1] With a few exceptions the lines from the same donor exhibited the same genetic profile.

Losses of single alleles at individual loci were fairly common in the cell lines. This pattern of allelic loss is consistent with prior karyotype studies,[4,34–36] and loss of heterozygosity studies with these cell lines that revealed frequent losses of individual chromosome arms.[37–39] In some cases we noted loss of an allele in 1 but not both of the cell lines derived from the same donor. For example, in UM-SCC-17A and -17B, allele 17 at D18S51 was lost in UM-SCC-17B but not in UM-SCC-17A. UM-SCC-81A and -81B are perhaps the most unlike each other of all of the paired sets. These cell lines were considered to be from 2 separate primary tumors of the same donor that arose 5 years apart: the first from the larynx and the second from the tonsil. In this pair there were differences at 7 loci, although the genotype of each is consistent with the same donor origin of the cell lines. The cell lines share at least 1 allele at each locus with a single exception. At AMEL UM-SCC-81A but not -81B lost the Y chromosome signal. At D3S1358, UM-SCC-81A has allele 15, -81B does not; at FGA -81B has allele 20, -81A does not; at D8S1779 -81B has 13, -81A does not; at D18S51 81A has 19, -81B does not; at D13S317 81B has 11, -81A does not. The most interesting difference was at D21S11, where -81A has 33.2, whereas -81B has 29. We suspect that the donor's normal complement was allele 29, and 33.2 at this locus, but each tumor lost a different allele.

Genetic drift over time in cultured cell lines has been raised as a major concern for scientists using established cell lines. We had previously assessed the karyotype of cultured SCC cell lines over numerous passages and found remarkable stability.[34] In the present study, comparison of allelic patterns in 3 different cell lines taken at low passage and >50 passages revealed no changes in the distribution of alleles, suggesting stability at each locus (Table 2). However, in high-passage UM-SCC-1, allele amplicons for AMEL-Y and FGA-22 were lost, and, in high-passage UM-SCC-22A, 1 wVA-15 allele was lost.

To further characterize the ability of this assay to discriminate genotypes between cancer cell lines and normal human fibroblasts, we genotyped short-term cultured fibroblasts from the donors of UM-SCC-11, -26, and -42, and then we

Table 1. Genotyping results for 73 UM-SCC cell lines.

Cell line	Sex	Specimen site	Primary location	Passage	AMEL	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
UM-SCC-1	M	Floor of mouth	Floor of mouth	4	X, Y	18	15, 18	18, 22	13, 16	27	18	10, 13	8, 11	9, 12
UM-SCC-2	F	Alveolar ridge	Alveolar ridge	6	X	16	16	19	12, 14	30, 33.2	18	12	11, 13	8, 12
UM-SCC-3	F	Lymph node	Nasal	6	X	17	17	20, 27	14	31.2	12, 15	11, 13	11, 13	9, 10
UM-SCC-4	F	BOT	Tonsillar pillar	7	X	17	17, 18	26	13, 15	28	16	11	12	9, 10
UM-SCC-5	M	Supraglottis	Supraglottis	20	X	17	18	25	11, 13	31	12, 16	13	8, 11	9
UM-SCC-6	M	BOT	BOT	22	X, Y	15	15, 16	23	11, 14	28	11, 19	12	13	10, 11
UM-SCC-7	M	Alveolus	Alveolus	23	X, Y	15	16	20, 22	12, 15	33.2	14, 15	11, 12	10	8, 12
UM-SCC-8	F	Alveolus	Alveolus	9	X	14, 16	16	22, 23	10, 13	29, 31.2	13, 15	11, 12	12	10, 11
UM-SCC-9	F	Anterior tongue	Anterior tongue	3	X	14	17, 18	22	9, 13	30, 31.2	13, 15	12	11, 14	8, 11
UM-SCC-10A	M	True vocal cord	True vocal cord	80	X	17	19	22, 26	13, 14	30	15	12, 13	9	9
UM-SCC-10B	M	Lymph node	Larynx	24	X	17	19	22	13, 14	30	15	12, 13	9	9
UM-SCC-11A	M	Epiglottis	Epiglottis	10	X	16	16, 17, 18	19, 24	12, 15	28	16	11	14	11
UM-SCC-11B	M	Supraglottic larynx	Supraglottic larynx	38	X	16	16, 17, 18	19	15	28	16	11	14	11
UM-SCC-12	M	Larynx	Larynx	77	X	15	16	23, 25	14, 15	29	12, 16	12	13	10, 11
UM-SCC-13	M	Esophagus	Larynx	21	X	16, 18	17	19, 24	12, 14	27, 28	14, 16	13	12	8, 12
UM-SCC-14A	F	Floor of mouth	Floor of mouth	32	X	15	14, 18	20, 21	8, 13	29	15	11, 14	12	9, 10
UM-SCC-14B	F	Floor of mouth	Floor of mouth	8	X	15	14, 18	20, 21	8, 13	29	15	11, 14	12	9, 10
UM-SCC-14C	F	Floor of mouth	Floor of mouth	5	X	15	14, 18	20, 21	8, 13	29	15	11, 14	12	9, 10
UM-SCC-15*	M	Hypopharynx	Hypopharynx	3	X	18	17	23	13, 14	31, 31.2	14	12	12	9
UM-SCC-16	F	Larynx	Larynx	2	X	14	15, 17	21, 24	10, 14	28, 29.2	15	10, 12	8, 10	10, 12
UM-SCC-17A	F	Supraglottis	Supraglottis	22	X	15, 18	14, 17	20, 22	12, 13	28	17, 22	11	11, 13	13
UM-SCC-17B	F	Soft tissue–neck	Supraglottis	33	X	15, 18	14, 17	20, 22	12, 13	28	22	11	11, 13	13
UM-SCC-18*	M	BOT	BOT	25	X	13, 14	15, 19	19, 20	14, 15	29, 30	14	11	9, 12	9
UM-SCC-19	M	BOT	BOT	4	X, Y	14	16, 19	24	10, 12	28, 30	10, 16	11	11	9
UM-SCC-20*	M	Neck node	Larynx	2	X, Y	14, 15	16, 18	18, 22.2	12, 15	28, 30.2	13, 15	11, 12	8, 10	9, 10
UM-SCC-21A	M	Ethmoid sinus	Skin of nose	56	X, Y	15	16, 18	21	13, 14	29, 32.2	14	11, 12	8, 9	10, 11
UM-SCC-22A	F	Hypopharynx	Hypopharynx	16	X	16	15, 18	22, 24	11, 13	28	18	12	8, 12	8, 9
UM-SCC-22B	F	Neck metastasis	Hypopharynx	18	X	16	15, 18	22, 24	11, 13	28	18	12	8, 12	8, 9
UM-SCC-23	F	Larynx	Larynx	47	X	17	17	20	10, 15	29	10	11, 12	8	8, 13
UM-SCC-24	M	Larynx	True vocal cord	5	X, Y	17	16, 17	18, 25	13	32.2	15	13	11, 12	9
UM-SCC-25	M	Neck	Larynx	41	X, Y	16	19	21	13, 14	27	19	12	11	12, 13
UM-SCC-26	M	Neck	BOT	10	X, Y	16	16, 17	21, 24	13, 15	32.2	14	10, 11	11	7, 12
UM-SCC-27*	M	Neck	Anterior tongue	4	X, Y	14	17, 18	19	12, 13	29, 32.2	14, 16	12, 13	8?	8, 11
UM-SCC-28	F	True vocal cord	True vocal cord	5	X	15	18, 19	23, 25	13, 15	30, 32.2	14	12	12	11
UM-SCC-29	M	Alveolis	Alveolis	18	X	14	18, 19	23, 25	15	28	15	11	8, 11	8, 10
UM-SCC-30	M	Pyriform sinus	Pyriform sinus	15	X	16	16, 17	21	14, 15	29	14	12	13	9, 10
UM-SCC-31	M	Tonsil	Tonsil	9	X	16	17, 18	24	14	30	13, 18	12	10, 12	8
UM-SCC-33	M	Neck	Maxillary sinus	16	X	17	14, 18	19	12	31	18	10	11	10
UM-SCC-34	M	Tonsillar pillar	Tonsillar pillar	11	X, Y	15	15, 18	23	9, 14	30, 31	13	12, 13	12, 13	10, 12
UM-SCC-35	M	Tonsillar fossa	Tonsillar fossa	8	X, Y	17	15, 18	18.2, 26	11, 15, 16	36	15	13	11	9, 11
UM-SCC-36	M	False vocal cord	False vocal cord	11	X, Y	17	17, 18	21	12, 13	30	20	11	11, 12	9, 11
UM-SCC-37	M	Vallecula	Vallecula	12	X	15	18, 19	20, 22	11, 14	30	14, 17	11, 12	10	9, 11

(Continued)

Table 1. Genotyping results for 73 UM-SCC cell lines (Continued).

Cell line	Sex	Specimen site	Primary location	Passage	AMEL	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
UM-SCC-38	M	Tonsillar pillar	Tonsillar pillar	28	X, Y	18	17, 18	28	14, 15	27, 29	19	12	10, 11	10
UM-SCC-39	M	Pyriform sinus	Pyriform sinus	7	X, Y	16	17, 18	24	14	32.2	16, 17	11	13	10, 11
UM-SCC-40	M	Esophagus	Esophagus	10	X	14	14, 16	21, 22	13	28, 30	12	10	10	8, 10
UM-SCC-41	M	Arytenoid	Arytenoid	9	X	16	18	25	14	29	18	12	11	10, 12
UM-SCC-42	M	Neck	Pyriform sinus	7	X	16	18	19, 20	10, 14	29, 30	12	11, 12	13	11, 12
UM-SCC-43	M	Palate	Palate	10	X, Y	18	14, 15	20, 22	13, 15	32.2	14, 16	11	11, 13	10, 12
UM-SCC-44	M	Neck	Retromolar trigone	13	X	17	16, 17	24	13	30	12, 13	12	12	9, 13
UM-SCC-45	F	Neck	Floor of mouth	6	X	17	14, 16	21	11, 14	28, 30	19	12	10	9, 10
UM-SCC-46	F	Larynx	Larynx	3	X	15	14, 18	21	13, 15	30, 31.2	19	10, 11	10	12, 14
UM-SCC-47	M	Lateral tongue	Lateral tongue	29	X, Y	15	18	23, 25	15	29, 30	18	11, 12	8, 11	11
UM-SCC-48	M	Neck	Retromolar trigone	5	X	14	17	18, 21	13, 14	29, 31.2	15	12, 13	12	8, 11
UM-SCC-49	M	Lateral tongue	Lateral tongue	1	X	15	16, 20	23, 26	12	27	15	11	12, 13	8, 9
UM-SCC-50	M	BOT	BOT	7	X	15	16, 18	21, 22	13, 15	29	17	9, 12	11	10, 12
UM-SCC-51	M	Floor of mouth	Floor of mouth	12	X	16	14, 15	20, 21	9, 12	29	14	12	11	13
UM-SCC-52	F	Supraglottic larynx	Supraglottic larynx	10	X	16	16, 18, 19	19	13, 16,17	30	12	13	13	9, 12
UM-SCC-53	M	Tonsil	Pyriform sinus	9	X	14	17	24	12, 15	27, 30	14, 21	12	8	10
UM-SCC-54	M	Larynx	True vocal cord	5	X, Y	15, 16	14, 17	24, 25	12, 14	29	14, 15	12	10, 11	10, 12
UM-SCC-55	M	Tonsil	Retromolar trigone	12	X, Y	17	17	22, 24	12, 14	30, 31	16	12, 13	8, 13	10
UM-SCC-57	M	Supraglottic larynx	Supraglottic larynx	6	X, Y	15	16, 17	23, 24	12	29	17, 18	11, 12	9, 12	9, 10
UM-SCC-58	F	Supraglottic larynx	Supraglottic larynx	8	X	14	15, 16, 17	22, 25	13, 15	30, 32.2	13	11	9, 13	9, 11
UM-SCC-59	F	Lateral tongue	Lateral tongue	11	X	14	15	22	13, 14	28, 29	12	10	10	11, 12
UM-SCC-60	M	Hypopharynx	Hypopharynx	15	X, Y	16	14, 15	19, 26	11	32	12	11	14	9, 10
UM-SCC-69	M	Hard palate	Hard palate	17	X	17, 18	16, 17	20, 24	10, 14	30, 32.2	15, 16	11, 12	11	11, 13
UM-SCC-70	M	Larynx	Larynx	8	X, Y	14	16, 17	20	14	30	18	11	11	12
UM-SCC-73B	M	Neck	Tongue	9	X	16	16, 19	21	13, 15	30	12, 14	12	11	8, 9
UM-SCC-74A	M	BOT	BOT	14	X	15, 16	15, 16	21, 26	12, 13	30, 34.2	17	12	12	11
UM-SCC-74B	M	Intraoral	Larynx	4	X	15, 16	15, 16	21, 26	12, 13	30, 34.2	17	12	12	11
UM-SCC-80	M	Hypopharynx	Hypopharynx	12	X, Y	17	14, 17	22	13	31.2	24	9	11	10
UM-SCC-81A	M	L false vocal cord	Larynx	7	X	15, 17	17	22	10	33.2	14, 19	11, 12	9	9, 11
UM-SCC-81B	M	Tonsillar pillar	Tonsil	18	X, Y	17	17	20, 22	10, 13	29	14	11, 12	9, 11	9, 11
UM-SCC-92	F	Lateral tongue	Lateral tongue	16	X	15	17, 20	19, 21	10, 13	29, 31	14, 15	11	12, 14	11, 12

Abbreviations: UM-SCC, University of Michigan Squamous Cell Carcinoma cell line series; M, male; F, female; BOT, base of tongue.
Note: For each cell line the donor sex, the specimen site, the primary tumor location, the passage number of the cells genotyped for this study, and the alleles at 10 different tetranucleotide short tandem repeat loci (AMEL, D31358, vWA, FGA, D8S1179, D21S51, D18S51, D5S818, D13S317, and D7S820) are shown. Patients with heterozygous alleles for each locus have 2 numbers corresponding to the different alleles. Where only a single allele is listed, either the patient had homozygous alleles at the given locus, an allele was not amplified (false negative), or was lost from tumor chromosome instability cloned out in the process of cell line establishment. In all cases, the lowest passage culture of UM-SCC cell lines available was used for analysis.

Table 2. Genotyping results after long-term cell culture.

Cell line	Passage	AMEL	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
UM-SCC-1	4	X, Y	18	15, 18	18, 22	13, 16	27	18	10, 13	8, 11	9, 12
UM-SCC-1	>150	X	18	15, 18	18	16	27	18	10, 13	8, 11	9, 12
UM-SCC-2	6	X	16	16	19	12, 14	30, 33.2	18	12	11, 13	8, 12
UM-SCC-2	62	X	16	16	19	12, 14	30, 33.2	18	12	11, 13	8, 12
UM-SCC-22A	16	X	16	15, 18	22, 24	11, 13	28	18	12	8, 12	8, 9
UM-SCC-22A	138	X	16	18	22, 24	11, 13	28	18	12	8, 12	8, 9

Abbreviation: UM-SCC, University of Michigan Squamous Cell Carcinoma cell line series.
Note: Genotyping results for 3 UM-SCC cell lines at high and low passages demonstrate that alleles may be lost as a result of in vitro evolution of the population.

compared the results to the genotypes of the cancer cell lines. As shown in Table 3, many of the alleles that were lost during either malignant transformation of cell culture were present in the fibroblast line. For example, UM-SCC-11A has 7 loci that appear to have either homozygous or lost alleles and UM-SCC-11B appears to have 9 loci with only a single marker. However, genotyping of the donor fibroblast line revealed that only D21S11 has a single allele. Thus, only this allele is potentially homozygous or lost during culture. Analysis of the fibroblast data reveals that the UM-SCC cancer cell lines occasionally gain or lose a single allele at various loci. For example, in both UM-SCC-26 and UM-SCC-42, 4 alleles are lost at 4 different loci in each cancer cell line compared with the donor fibroblast line.

Representative photomicrographs of UM-SCC cell lines are shown in Figure 1 to illustrate the various in vitro morphologies typically exhibited by individual cell lines. Additional photographs of UM-SCC cell lines are also reported in 2 book chapters for comparison.[40,41] Note that changes occur with increasing cell density in some cell lines. For example, UM-SCC-5 and UM-SCC-17A grow as tightly packed colonies. UM-SCC-17B has a morphology similar to that of UM-SCC-17A, but the cells are less inclined to pack tightly, especially shortly after passage. UM-SCC-74A and -74B are from a patient previously treated with chemotherapy, and radiation and the cells in both cultures have undergone epithelial-mesenchymal transition, giving the culture a fibroblastoid appearance. This is consistent with the sarcomatoid morphology sometimes observed in tissue samples from recurrent SCC after radiation.

It was not possible to retrieve viable isolates for some of the original UM-SCC cell lines from liquid nitrogen storage. However, we did genotype the DNA from these nonviable cells so that if others have healthy cultures of the UM-SCC cell lines no longer available in our bank, the correct genotype of the original cell line is provided. Such examples (UM-SCC-15, -20, and -27) are marked in Table 1 with an asterisk. We discovered several examples of mislabeled cell lines within our own bank. However, for each of the mislabeled cell lines, we retrieved early-passage vials from our bank and found unique genotypes for each cell line. These were expanded and used to repopulate the tissue core bank.

Table 3. Genotyping results of 3 fibroblast and cancer cell lines from matched donors.

Cell line	Passage	AMEL	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
UM-SCC-11 fibroblasts	1	X, Y	14, 16	16, 17	19, 24	12, 15	28	16, 18	11, 12	12, 14	10, 11
UM-SCC-11A	10	X	16	16, 17, 18	19, 24	12, 15	28	16	11	14	11
UM-SCC-11B	38	X	16	16, 17, 18	19	15	28	16	11	14	11
UM-SCC-26 fibroblasts	2	X, Y	16, 17	16, 17	21, 24	13, 15	31, 32.2	14, 18	10, 11	11, 12	7, 12
UM-SCC-26	10	X, Y	16	16, 17	21, 24	13, 15	32.2	14	10, 11	11	7, 12
UM-SCC-42 fibroblasts	7	X, Y	15, 16	18	19, 20	10, 14	29, 30	12, 15	11, 12	12, 13	11, 12
UM-SCC-42	7	X	16	18	19, 20	10, 14	29, 30	12	11, 12	13	11, 12

Abbreviation: UM-SCC, University of Michigan Squamous Cell Carcinoma cell line series.
Note: For the UM-SCC-11, -26, and -42 cell lines,we were able to grow and genotype fibroblasts from the donor. In each case, the matched genotypes are shown. Donors with heterozygous alleles for each locus have 2 numbers corresponding to different alleles. Where only a single allele is listed, either the patient had homozygous alleles at the given locus, an allele was not amplified (false negative), or was lost from tumor chromosome instability cloned out in the process of cell line establishment.

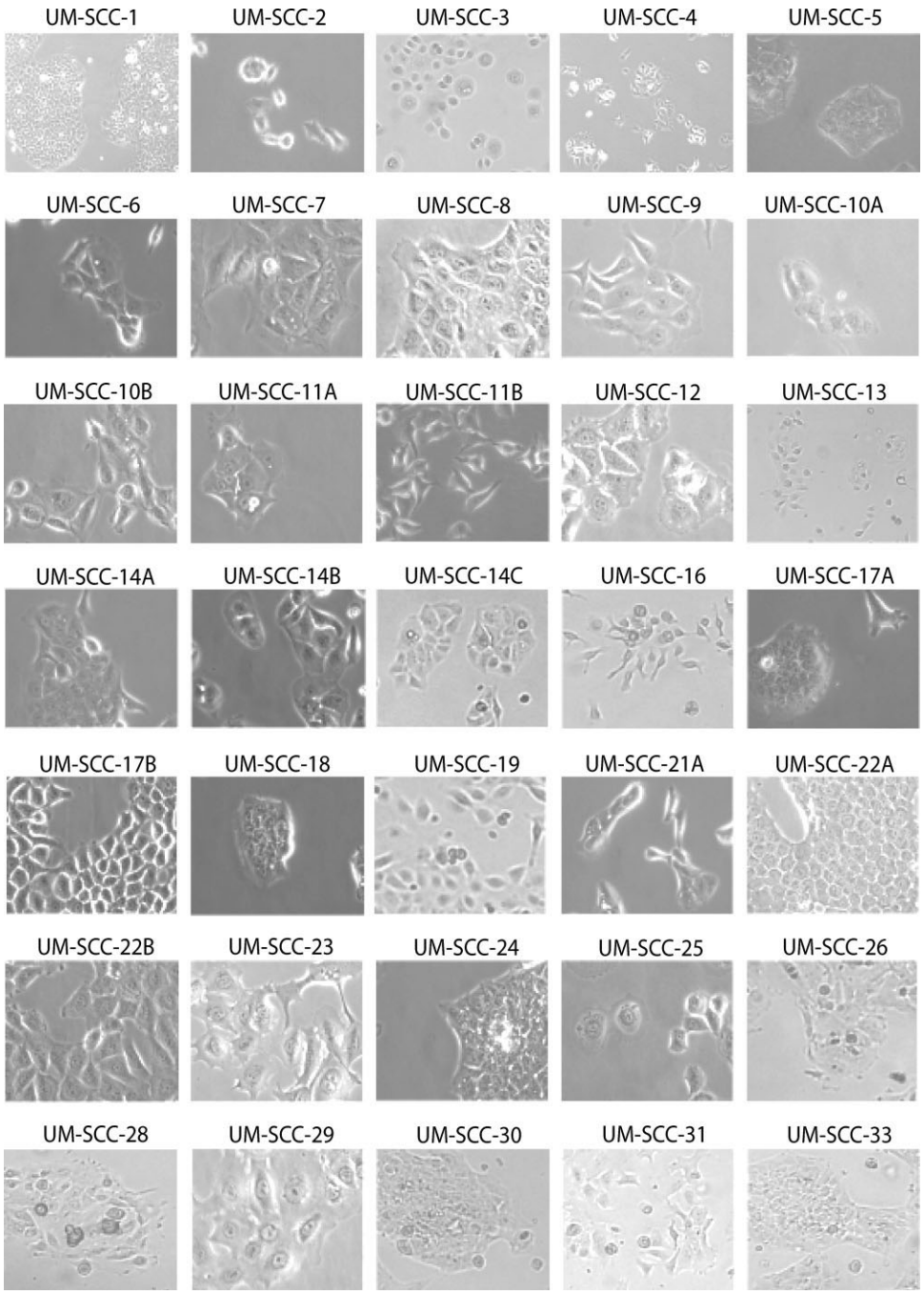

FIGURE 1. Representative photomicrographs. University of Michigan squamous cell carcinoma (UM-SCC) cell lines were cultured for 24 hours before photographs were captured under either a 10× (UM-SCC-1) or 40× objective lens (remaining cell lines). In all cases, genotyped cell lines were imaged.

DISCUSSION

A lack of vigilance in cell acquisition and identity testing has plagued scientific studies and publications since the inception of cell line methods.[42–47] In the 1970s and 1980s, examples of interspecies and intraspecies cross-contamination of human cell lines was documented by Nelson-Rees.[43,45] Data produced from cross-contaminated heterogeneous populations of cells, or incorrectly identified cell lines that might be from a different tumor type or even the wrong species, lead to incorrect conclusions,

experimental results that are not representative of a particular tumor or tissue type, confusion in the literature, and a general mistrust of data produced with cell lines. In 2004, for example, 1 study reported that 9% of 483 researchers used cultures containing HeLa contaminants.[49] Additionally, we performed a simple literature search for scientific papers that compared parental MCF-7 cells with an adriamycin-resistant cell line thought to be derived from MCF-7, called MCF-7/Adr. This search revealed 187 different papers, some of which have gone on to propose the use of novel chemotherapeutics in specific patient populations. However, it has recently been shown that MCF-7/Adr is actually an ovarian carcinoma cell line,[48] meaning that most of the data analysis between 2 cell lines is completely invalid.

Despite the critical nature of correctly identified cell lines as model systems, it has been difficult to get funding for cell line characterization, leaving researchers who realize the importance of the problem in the dark. The problem of contaminated cell lines has been addressed previously by 1 of us[40] as well as by others in more recent editorial articles in *Science* and several other journals.[20–22] A recent study[16] examined reports in the literature of the *TP53* mutational status from different investigators who studied the cells that are included in the National Cancer Institute panel of 60 representative human tumor cell lines. The authors reported finding discrepancies in the reported *TP53* mutation status for 13 of 60 cell lines (22%) included in this important repository. Their findings suggest that different versions of the cell lines are being used in various laboratories and that they may not be the cell line the investigators think they are using.

Because it is necessary to reliably genotype cells that have been cultured in independent laboratories for multiple years, several studies have focused on the reproducibility of microsatellite genotyping by studying long-term microsatellite stability. For example, Masters et al[23] analyzed HeLa cells that had been cultured independently by different labs over several years and found both gains and losses of alleles. However, only a few alleles were altered in each case, and, because of the consistency between the other alleles, the cell lines were still able to be identified as HeLa with very high probability. Likewise, the group analyzed the genotypes of cell lines derived by in vitro selection by long-term exposure to chemotherapy, and found that the differences between the STR loci were no greater than those between HeLa cells that had been independently cultured.[23] Despite the fact that cell lines can be identified after long periods of independent culturing, phenotypic differences arise in different laboratories because cell lines evolve in vitro, likely leading to the increased growth potential. As such, cell lines should be periodically refreshed from the low-passage stocks.

With the intense demand for the UM-SCC head and neck cancer cell lines from colleagues around the world, and a desire to ensure that results from multiple labs could be compared, we took advantage of the availability of rapid, low-cost, highly polymorphic microsatellite analysis to genotype our entire University of Michigan cell line panel. Like others before us, we were chagrined to find that over time mistakes had been made and mislabeling of cell lines had occurred even within our own cell line bank. Because ours is a laboratory that stresses good principles of tissue culture, this example shows how easily mistakes can be made and perpetuated in cell culture studies. Table 1 from this article and representative photographs of each of our generically characterized cell lines will be posted on the University of Michigan Head and Neck Cancer SPORE (Specialized Project of Research Excellence) web page for easy access for other investigators who have these lines in their laboratory. See also Supplemental Material.

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