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Genotyping of 73 UM-SCC head and neck squamous cell carcinoma cell lines

J. Chad Brenner, MSE[1,2], **Martin P. Graham, BS**[1], **Bhavna Kumar, MS**[1], **Lindsay M. Saunders, BS**[1], **Robbi Kupfer, MD**[1], **Robert H. Lyons, PhD**[3], **Carol R. Bradford, MD**[1], and **Thomas E. Carey, PhD**[1,2,4]

[1]Department of Otolaryngology-Head and Neck Surgery, University of Michigan, Ann Arbor, MI 48109

[2]Program in Cellular and Molecular Biology, University of Michigan, Ann Arbor, MI 48109

[3]DNA sequencing Core, University of Michigan, Ann Arbor, MI 48109

[4]Pharmacology, University of Michigan, Ann Arbor, MI 48109

Abstract

Background—We established multiple UM-SCC (University of Michigan Squamous Cell Carcinoma) 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.

Design—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 U of M 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.

Introduction

Head and neck squamous cell cancers (HNSCC) account for 11,170 deaths annually in the United States and nearly 250,000 deaths annually world wide. 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 UM-SCC cell lines developed at the University of Michigan 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)], as well as 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)], sequencing of hypervariable mitochondrial sequences[(15)], and some groups have even suggested *TP53* sequencing[(16)] since 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, 45/252 (18%) novel cell lines collected in the German Cell Line Bank were found to have non-unique 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, which lead to increased microsatellite instability[24] and prevent reliable genotyping. Microsatellites are short tandem repeat (STR) loci that are highly polymorphic repetitive DNA sequence elements 2–7 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 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 following separation of the different alleles by electrophoresis. Many of these loci are made up of dinucleotides 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 dinucleotide repeats, provide 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 10 common tetranucleotide repeat sequences on 73 of the most commonly used 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) 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 according to the manufacturer's protocol, and testing was repeated at monthly intervals.

Genomic DNA Purification

Exponentially growing (60–80% confluence) cells were trypsinized and washed in PBS. Cell pellets were flash frozen at −80°C, resuspended in 500μL of 0.1M Tris pH 8.0, 0.1M EDTA (ethylene diamine tetraacetic acid), 0.4M NaCl, 1% SDS (sodium dodecylsulfate) and 0.3mg/mL Proteinase K (NEB, Ipswich, MA and incubated overnight at 55°C). Following incubation, 500μL 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 150μL of 7.5M ammonium acetate and 800μL 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 HPLC grade H_2O.

Analysis of Genetic Loci

DNA samples were diluted to 0.10ng/μl and analyzed in the University of Michigan DNA sequencing Core using the Profiler Plus PCR (polymerase chain reaction) Amplification Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. The 9 loci D3S1358, D5S818, D7S820, D8S1179, D13S317, D18S51, D21S11, FGA, vWA and the amelogenin locus were analyzed and compared to ladder control samples.

Results

Genetic Profiling of UM-SCC cell lines

For each of the genotyped cell lines now represented in the U of M Head and Neck SPORE cell line bank, the UM-SCC cell line number, the donor gender, 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 gender identification, however, since 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, presence of a Y signal was only observed 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 more than one 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 one 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 two separate primary tumors of the same donor that arose 5 years apart; one 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 one allele at each locus with one 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 three different cell lines taken at low passage and greater than 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, one 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 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 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, four alleles are lost at four different loci in each cancer cell line as compared to 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 two 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 similar morphology to 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 who was 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 non-viable 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.

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 80s examples of inter 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 leads 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, one study reported that 9% of 483 researchers used cultures containing HeLa contaminants.[48] 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 and 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[49] meaning that most of the data analysis between two 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 one of us[40] as well as in more recent editorial articles in *Science* and several other journals.[20–22] A recent paper[16] examined reports in the literature of the *TP53* mutational status from different investigators who studied the cells that are included in the NCI panel of 60 representative human tumor cell lines. The authors reported finding discrepancies in the reported *TP53* mutation status for 13/60 (22%) cell lines included this important repository. Their findings suggest that different version 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 insure 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. Since 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 paper and representative photographs of each of our genetically characterized cell lines will be posted on the University of Michigan Head and Neck Cancer SPORE web page for easy access for other investigators who have these lines in their laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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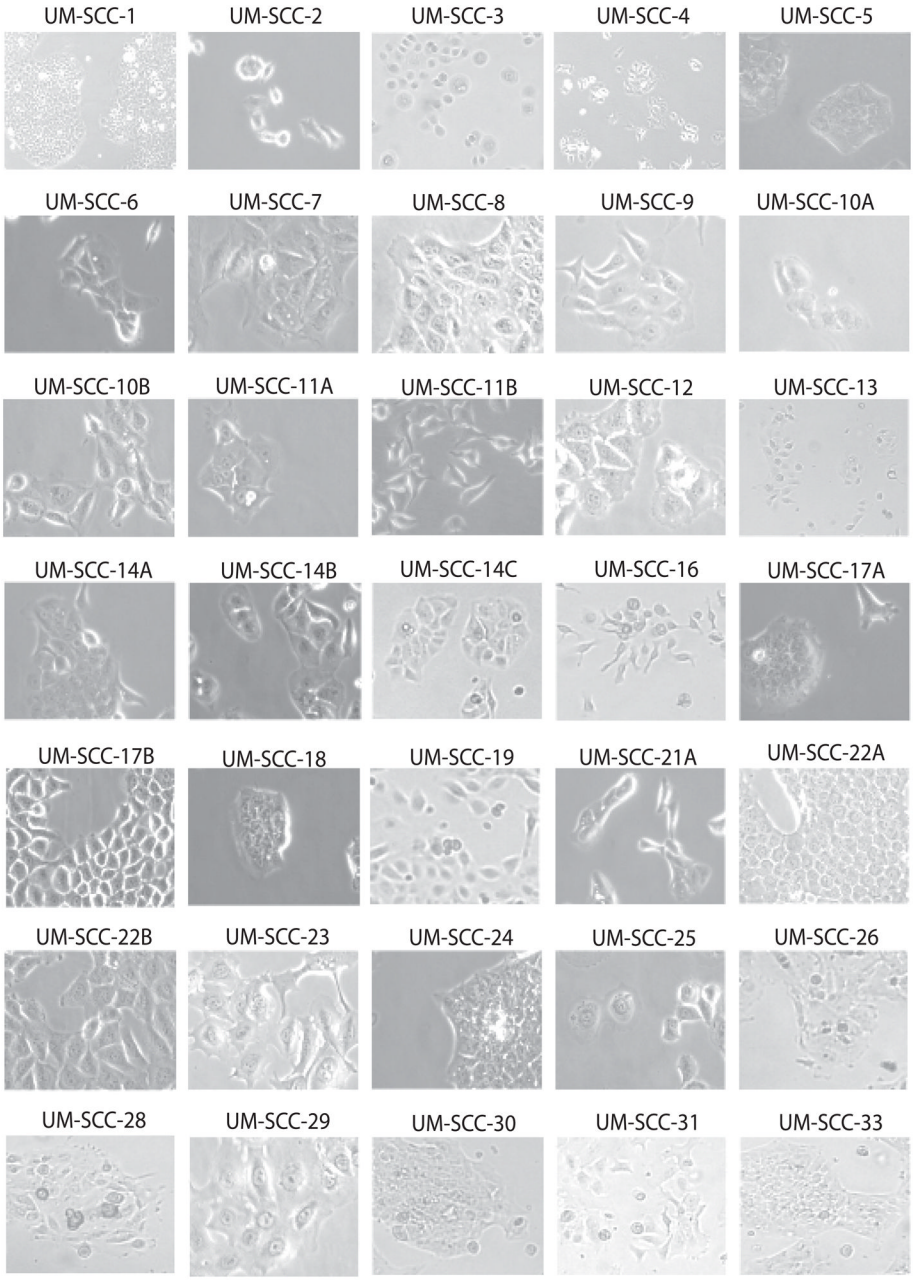

Figure 1. Representative photomicrographs

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

Table 1

Genotyping results for 73 UM-SCC cell lines

Patients with heterozygous alleles for each locus have two 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. Genotypes of 73 UM-SCC cell lines established from 65 donors. 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. Abbreviations: UM-SCC, University of Michigan Squamous Cell Carcinoma cell line series; M, male; F, female; BOT, base of tongue.

CELL LINE	Gender	Specimen Site	Primary Location	Passage	AMEL	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
UM-SCC-1	Male	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	Female	alveolar ridge	alveolar ridge	6	X	16	16	19	12, 14	30, 33.2	18	12	11, 13	8, 12
UM-SCC-3	Female	lymph node	nasal	6	X	17	17	20, 27	14	31.2	12, 15	11, 13	11, 13	9, 10
UM-SCC-4	Female	BOT	tonsillar pillar	7	X	17	17,18	26	13, 15	28	16	11	12	9,10
UM-SCC-5	Male	Supraglottis	Supraglottis	20	X	17	18	25	11, 13	31	12,16	13	8,11	9
UM-SCC-6	Male	BOT	BOT	22	X, Y	15	15, 16	23	11, 14	28	11, 19	12	13	10, 11
UM-SCC-7	Male	alveolus	alveolus	23	X, Y	15	16	20, 22	12, 15	33.2	14, 15	11, 12	10	8, 12
UM-SCC-8	Female	alveolus	alveolus	9	X	14, 16	16	22, 23	10, 13	29, 31.2	13, 15	11, 12	12	10, 11
UM-SCC-9	Female	ant. tongue	ant. tongue	3	X	14	17, 18	22	9, 13	30, 31.2	13, 15	12	11, 14	8, 11
UM-SCC-10A	Male	true vocal cord	true vocal cord	80	X	17	19	22, 26	13, 14	30	15	12, 13	9	9
UM-SCC-10B	Male	lymph node	larynx	24	X	17	19	22	13,14	30	15	12,13	9	9
UM-SCC-11A	Male	epiglottis	epiglottis	10	X	16	16, 17, 18	19, 24	12, 15	28	16	11	14	11
UM-SCC-11B	Male	supraglottic larynx	supraglottic larynx	38	X	16	16, 17, 18	19	15	28	16	11	14	11
UM-SCC-12	Male	larynx	larynx	77	X	15	16	23, 25	14, 15	29	12, 16	12	13	10, 11
UM-SCC-13	Male	esophagus	larynx	21	X	16,18	17	19,24	12, 14	27,28	14, 16	13	12	8,12
UM-SCC-14A	Female	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	Female	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	Female	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*	Male	hypopharynx	hypopharynx	3	X	18	17	23	13, 14	31, 31.2	14	12	12	9
UM-SCC-16	Female	larynx	larynx	2	X	14	15, 17	21, 24	10, 14	28, 29.2	15	10, 12	8, 10	10, 12
UM-SCC-17A	Female	supraglottis	supraglottis	22	X	15, 18	14, 17	20, 22	12, 13	28	17, 22	11	11, 13	13

CELL LINE	Gender	Specimen Site	Primary Location	Passage	AMEL	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
UM-SCC-17B	Female	soft tissue-neck	supraglottis	33	X	15, 18	14, 17	20, 22	12, 13	28	22	11	11, 13	13
UM-SCC-18	Male	BOT	BOT	25	X	13, 14	15, 19	19, 20	14, 15	29, 30	14	11	9, 12	9
UM-SCC-19	Male	BOT	BOT	4	X, Y	14	16, 19	24	10, 12	28, 30	10, 16	11	11	9
UM-SCC-20*	Male	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	Male	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	Female	hypopharynx	hypopharynx	16	X	16	15, 18	22, 24	11, 13	28	18	12	8, 12	8, 9
UM-SCC-22B	Female	neck metastasis	hypopharynx	18	X	16	15, 18	22, 24	11, 13	28	18	12	8, 12	8, 9
UM-SCC-23	Female	larynx	larynx	47	X	17	17	20	10, 15	29	10	11, 12	8	8, 13
UM-SCC-24	Male	larynx	true vocal cord	5	X, Y	17	16, 17	18, 25	13	32.2	15	13	11, 12	9
UM-SCC-25	Male	neck	larynx	41	X, Y	16	19	21	13, 14	27	19	12	11	12, 13
UM-SCC-26	Male	neck	BOT	10	X, Y	16	16, 17	21, 24	13, 15	32.2	14	10, 11	11	7, 12
UM-SCC-27	Male	neck	ant. tongue	4	X, Y	14	17, 18	19	12, 13	29, 32.2	14, 16	12, 13	8?	8, 11
UM-SCC-28	Female	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	Male	alveolus	alveolus	18	X	14	18, 19	23, 25	15	28	15	11	8, 11	8, 10
UM-SCC-30	Male	pyriform sinus	pyriform sinus	15	X	16	16, 17	21	14, 15	29	14	12	13	9, 10
UM-SCC-31	Male	tonsil	tonsil	9	X	16	17, 18	24	14	30	13, 18	12	10, 12	8
UM-SCC-33	Male	neck	maxillary sinus	16	X	17	14, 18	19	12	31	18	10	11	10
UM-SCC-34	Male	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	Male	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	Male	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	Male	vallecula	vallecula	12	X	15	18, 19	20, 22	11, 14	30	14, 17	11, 12	10	9, 11
UM-SCC-38	Male	tonsillar pillar	tonsillar pillar	28	X, Y	18	17, 18	28	14, 15	27, 29	19	12	10, 11	10
UM-SCC-39	Male	pyriform sinus	pyriform sinus	7	X, Y	16	17, 18	24	14	32.2	16, 17	11	13	10, 11
UM-SCC-40	Male	esophagus	esophagus	10	X	14	14, 16	21, 22	13	28, 30	12	10	10	8. 10
UM-SCC-41	Male	arytenoid	arytenoid	9	X	16	18	25	14	29	18	12	11	10, 12
UM-SCC-42	Male	neck	pyriform sinus	7	X	16	18	19, 20	10, 14	29, 30	12	11, 12	13	11, 12
UM-SCC-43	Male	palate	palate	10	X, Y	18	14, 15	20, 22	13, 15	32.2	14, 16	11	11, 13	10, 12
UM-SCC-44	Male	neck	retromolar trigone	13	X	17	16, 17	24	13	30	12, 13	12	12	9, 13
UM-SCC-45	Female	neck	floor of mouth	6	X	17	14, 16	21	11, 14	28, 30	19	12	10	9, 10

CELL LINE	Gender	Specimen Site	Primary Location	Passage	AMEL	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
UM-SCC-46	Female	larynx	larynx	3	X	15	14, 18	21	13, 15	30, 31.2	19	10, 11	10	12, 14
UM-SCC-47	Male	lateral tongue	lateral tongue	29	X, Y	15	18	23, 25	15	29, 30	18	11, 12	8, 11	11
UM-SCC-48	Male	neck	retromolar trigone	5	X	14	17	18, 21	13, 14	29, 31.2	15	12, 13	12	8, 11
UM-SCC-49	Male	lateral tongue	lateral tongue	1	X	15	16, 20	23, 26	12	27	15	11	12, 13	8, 9
UM-SCC-50	Male	BOT	BOT	7	X	15	16, 18	21, 22	13, 15	29	17	9, 12	11	10, 12
UM-SCC-51	Male	floor of mouth	floor of mouth	12	X	16	14, 15	20, 21	9, 12	29	14	12	11	13
UM-SCC-52	Female	supraglottic larynx	supraglottic larynx	10	X	16	16, 18, 19	19	13, 16, 17	30	12	13	13	9, 12
UM-SCC-53	Male	tonsil	pyriform sinus	9	X	14	17	24	12, 15	27, 30	14, 21	12	8	10
UM-SCC-54	Male	larynx	true vocal cord	43	X	16	17	17	14	29	16, 18	10, 12	12	10, 11
UM-SCC-55	Male	tonsil	retromolar trigone	12	X, Y	17	17	22, 24	12, 14	30, 31	16	12, 13	8, 13	10
UM-SCC-56	Male	penis	penis	11	X, Y	17	14,16	22,25	14	28,31	14	11,12	8,14	8,10
UM-SCC-57	Male	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	Female	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	Female	lateral tongue	lateral tongue	11	X	14	15	22	13, 14	28, 29	12	10	10	11, 12
UM-SCC-60	Male	hypopharynx	hypopharynx	15	X, Y	16	14, 15	19, 26	11	32	12	11	14	9, 10
UM-SCC-69	Male	hard palate	hard palate	17	X, Y	17, 18	16, 17	20, 24	10, 14	30, 32.2	15, 16	11, 12	11	11, 13
UM-SCC-70	Male	larynx	larynx	8	X	14	16, 17	20	14	30	18	11	11	12
UM-SCC-73B	Male	neck	tongue	9	X	16	16, 19	21	13, 15	30	12, 14	12	11	8, 9
UM-SCC-74A	Male	BOT	BOT	14	X	15, 16	15, 16	21, 26	12, 13	30, 34.2	17	12	12	11
UM-SCC-74B	Male	intraoral	larynx	4	X	15, 16	15, 16	21, 26	12, 13	30, 34.2	17	12	12	11
UM-SCC-80	Male	hypopharynx	hypopharynx	12	X, Y	17	14,17	22	13	31.2	24	9	11	10
UM-SCC-81A	Male	L false vocal cord	larynx	7	X	15, 17	17	22	10	33.2	14, 19	11, 12	9	9, 11
UM-SCC-81B	Male	tonsillar pillar	tonsil	18	X, Y	17	17	20,22	10,13	29	14	11,12	9,11	9,11
UM-SCC-92	Female	lateral tongue	lateral tongue	16	X	15	17, 20	19, 21	10, 13	29, 31	14, 15	11	12, 14	11, 12

Table 2

Genotyping results following long-term cell culture

Genotyping results for three UM-SCC cell lines at high and low passages demonstrate that alleles may be lost due as a result of in vitro evolution of the population.

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

Table 3

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

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 two 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.

	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