Mistaken Identity of Widely Used Esophageal Adenocarcinoma Cell Line TE-7

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Abstract
Cancer of the esophagus is the seventh leading cause of cancer death worldwide. Esophageal carcinoma cell lines are useful models to study the biological and genetic alterations in these tumors. An important prerequisite of cell line research is the authenticity of the used cell lines because the mistaken identity of a cell line may lead to invalid conclusions. Estimates indicate that up to 36% of the cell lines are of a different origin or species than supposed. The TE series, established in late 1970s and early 1980s by Nishihira et al. in Japan, is one of the first esophageal cancer cell line series that was used throughout the world. Fourteen TE cell lines were derived from human esophageal squamous cell carcinomas and one, TE-7, was derived from a primary esophageal adenocarcinoma. In numerous studies, this TE-7 cell line was used as a model for esophageal adenocarcinoma because it is one of the few esophageal adenocarcinoma cell lines existing. We investigated the authenticity of the esophageal adenocarcinoma cell line TE-7 by xenografting, short tandem repeat profiling, mutation analyses, and array-comparative genomic hybridization and showed that cell line TE-7 shared the same genotype as the esophageal squamous cell carcinoma cell lines TE-2, TE-3, TE-12, and TE-13. In addition, for more than a decade, independent TE-7 cultures from Japan, United States, United Kingdom, France, and the Netherlands had the same genotype. Examination of the TE-7 cell line xenograft revealed the histology of a squamous cell carcinoma. We conclude that the TE-7 cell line, used in several laboratories throughout the world, is not an adenocarcinoma, but a squamous cell carcinoma cell line. Furthermore, the cell lines TE-2, TE-3, TE-7, TE-12, and TE-13 should be regarded as one single squamous cell carcinoma cell line. [Cancer Res 2007;67(17):7996–8001]

Introduction
Cancer of the esophagus is the seventh leading cause of cancer death worldwide (1). Although esophageal squamous cell carcinoma cases have steadily declined, the incidence of esophageal adenocarcinoma has increased more rapidly than for any other cancer type and parallels increases in obesity and reflux disease (2). Despite the common occurrence of esophageal squamous cell carcinoma and esophageal adenocarcinoma, little is known about the molecular mechanisms underlying the genesis of these tumors. Tumor type–specific cell lines are useful models to study cell biological and molecular characteristics of the comparable tumors. Therefore, established tumor cell lines are of great importance, especially from tumor types from which only few lines exist. An important prerequisite of cell line research is the authenticity of the used cell lines because the mistaken identity of a cell line may lead to invalid conclusions (3). Estimates indicate that up to 36% of the cell lines are of a different origin or species from that being claimed (4–6). One of the major causes is cross-contamination between cell lines (see, e.g., refs. 3, 7). Cross-contamination of cell lines can be the result of poor culture technique when a cell line culture is contaminated with another cell line. Alternatively, cross-contamination of cell lines can be due to clerical error by mislabeling of cell cultures or frozen stocks.

The TE series, established by Nishihira et al. (8) and Kuriya et al. (9) in Japan, is one of the first esophageal cancer cell line series that was used throughout the world. Of the 15 TE cell lines generated, 14 were derived from human esophageal squamous cell carcinoma and one, TE-7, from a primary esophageal adenocarcinoma (10). This last cell line is one of the few esophageal adenocarcinoma cell lines generated and therefore frequently used as an in vitro model of esophageal adenocarcinoma throughout the world. The high incidence of cross-contamination prompted us to investigate the authenticity of the TE series and especially the TE-7 cell line by xenografting, short tandem repeat (STR) profiling, mutation analyses, and array-comparative genomic hybridization (array-CGH).

Materials and Methods
Cell cultures, DNA isolation, and xenografts. Cell lines TE-1 to TE-15, except TE-7, were obtained from the Japanese Cell Resource Center for Biomedical Research (Sendai, Japan). Cell line TE-7 was obtained from the originator Dr. T. Nishihira (Second Department of Surgery, Tohoku University, Sendai, Japan). Cells were cultured under standard conditions in RPMI 1640 supplemented with 10% FCS. To study the

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http://www.sanger.ac.uk/genetics/CGP/Genotyping/synlimestable.shtml
histologic characteristics of the TE-7 cell line. $5 \times 10^6$ trypsinized tumor cells were injected s.c. in female NMRI nude mice. Xenografts were removed and routinely processed for histologic and immunohistochemical examination. The animal experiments were licensed and done in accordance with approved protocols by the Erasmus MC, University Medical Center Rotterdam, the Netherlands. DNA was isolated from cultured cells and xenografts using the PureGene Genomic DNA isolation Kit (Qiagen). DNA was diluted to a concentration of 100 ng/μL to carry out PCR.

**STR profiling.** The cell lines TE-1 to TE-15 were genotyped by STR profiling using the Powerplex 16 System (Promega). The Powerplex 16 system is composed of 15 STR loci, including Penta E, D8S1179, TH01, D3S1338, FGA, TPOX, D8S1179, vWA, Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818, and the sex chromosome marker.

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**Figure 1.** Histology of xenograft TE-7. A, H&E staining showing a poorly differentiated squamous cell carcinoma, characterized by focal keratinization (magnification, ×40). B to D, immunostaining with involucrin, keratin 10, and keratin 14, respectively, showing strongly positive tumor cells (magnification, ×40).

**Figure 2.** STR profile of cell line TE-7. STR loci are indicated in boxes above electropherogram; numbers of repeat units are indicated below the peaks. A, Amelogenin.
Amelogenin. Amplification was done using 1 ng of template DNA applying the Powerplex 16 system following the manufacturer's recommendation. Multiplex PCR reactions were carried out by using fluorescent dye–linked primers. Labeled products were detected by electrophoretic size fractionation on an ABI 3100 genetic analyzer. The data were analyzed by using Genescan and Genotyper software (Perkin-Elmer) to categorize peaks according to their size in relation to an internal standard run. This analysis enabled every peak to be allocated a size corresponding to the number of repeat units present.

**Mutation analyses.** Because TP53 is frequently mutated in both esophageal adenocarcinoma and esophageal squamous cell carcinoma (11, 12), all exons and intron-exon boundaries of the TP53 gene were commercially sequenced in the TE-7 cell line (Asper Biotech Ltd.). The detected TP53 mutation in TE-7 was investigated in the other 14 cell lines by in-house sequencing at the Department of Pathology, Erasmus MC, University Medical Center Rotterdam. Primers used were **forward 5′-CAACGTTCTGGTAAGGACAAG-3′** and reverse **5′-GTGGGCACGTGGCCAAGATG-3′** for **TP53** exon 5 mutation ([Cyclin D1](http://example.com); refs. 13, 14). Both these DNA variants were investigated by bidirectional DNA sequencing of PCR products with the above-described procedure. PCR products were generated by standard procedures with primers forward **5′-GCACAAGACGGACCCCAAGG-3′** and reverse **5′-GACTGTCAGGGGAGCACCTG-3′** for **CYCLIN D1** and forward **5′-GTGGGACCGTGGCAGAGTG-3′** and reverse **5′-GCGTGTGCTGGTGGACGCTG-3′** for **AXIN1**.

**Array-CGH.** The array-based CGH procedure was done as previously described. Slides containing triplicates of ~3,500 large insert BAC clones spaced at density over the full genome were produced at the Leiden University Medical Center, Leiden, the Netherlands. The particular clone set used to produce these arrays is distributed to academic institutions by the University Medical Center, Leiden, the Netherlands. The particular clones set used to produce these arrays is distributed to academic institutions by the University Medical Center, Leiden, the Netherlands.

**Table 1. STR profiles of the TE series**

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Abbreviation: Amg, Amelogenin.

*Note the highly similar profiles in TE-2, TE-3, TE-7, TE-12, and TE-13.

Figure 3. Mutation analyses of cell line TE-7. A, homozygous TP53 splice site mutation TP53 NM_000546.3: c.375+1G>A in intron 4. B, heterozygous CYCLIN D1 exon 5 mutation [CCND1 NM_053056.2: c.859G>C(p.Pro287Ala)]. C, homozygous single-nucleotide polymorphism (SNP) in exon 6 of the AXIN1 gene [AXIN1 NM_1810850.1: c.1575C>T(p.Asp525Asp)(rs1805102); refs. 13, 14]. Both these DNA variants were investigated by bidirectional DNA sequencing of PCR products with the above-described procedure. PCR products were generated by standard procedures with primers forward **5′-GCACAAGACGGACCCCAAGG-3′** and reverse **5′-GACTGTCAGGGGAGCACCTG-3′** for **CYCLIN D1** and forward **5′-GTGGGACCGTGGCAGAGTG-3′** and reverse **5′-GCGTGTGCTGGTGGACGCTG-3′** for **AXIN1**.
Welcome Trust Sanger Institute\textsuperscript{10} at no cost and contains targets spaced at a density of $-1$ Mb over the full genome, a set of subtelomeric sequences for each chromosome arm, and a few hundred probes selected for their involvement in oncogenesis. After hybridization, the slides were scanned with a ScanArray Express HT (Perkin-Elmer Life Sciences) to collect 16-bit TIF images through Cy3 and Cy5 filter sets. The spot intensities were measured with GenePix Pro 5.1 software (Axon Instruments). Further analyses were done using Microsoft Excel 2000 (15, 16).

**Results and Discussion**

Human tumor cell lines are indispensable models to study molecular and cell biological characteristics of human tumors. For this, the correct identification of a tumor cell line is crucial because a mistaken identity of a cell line may lead to invalid conclusions with regard to the tumor type studied. Well-established methods to investigate the authenticity of tumor cell lines are STR profiling, SNP typing, mutation analyses, and karyotyping (3–5, 17). Using different methods, we show that cell line TE-7, widely used as an esophageal adenocarcinoma cell line, is a squamous cell carcinoma cell line. Furthermore, we prove that the human esophageal cancer cell lines TE-2, TE-3, TE-7, TE-12, and TE-13 are genotypically identical and therefore should be regarded as one single cell line.

Xenografting of cell line TE-7 resulted in a poorly differentiated squamous cell carcinoma (Fig. 1A). The xenografts were positive for cytokeratin 10, cytokeratin 14, and involucrin and negative for CDX2 expression, confirming the squamous histology (Fig. 1B–D). The genotypes of the TE-7 xenograft and in vitro cell line were identical showing that the xenograft tumor was derived from the

\textsuperscript{10}http://www.ensembl.org/
TE-7 cell line. STR profiling of the TE series revealed highly similar genotypes for the cell lines TE-2, TE-3, TE-7, TE-12, and TE-13 (Fig. 2; Table 1). However, at the D8S1179 and FGA loci, loss of one allele was seen in cell lines TE-7 and TE-3 and cell lines TE-7 and TE-13, respectively. These minor genetic changes are most likely due to genomic instability because these cancer cell lines were cultured apart for more than 20 years. Furthermore, loss of one allele at FGA and D8S1179 loci was also described in cross-contaminants of HeLa cells, indicating that allelic loss frequently occurs at these particular loci (4). Among other STR markers showed identical number of repeats in cell lines TE-2, TE-3, TE-7, TE-12, and TE-13 and provide strong evidence that these five cell lines are genotypically identical (Table 1). In addition, a homozygous TP53 splice site mutation TP53 NM_000546.3: c.375+1G>A in intron 4; the previously described study found that TE-7 cells clustered with the esophageal adenocarcinoma cell lines (18). Another gene expression profiling study revealed clustering of TE-7 with esophageal squamous cell carcinoma cell lines (19) because these five responding cell lines should be regarded as one single cell line. Another investigation showed that expression of PPARγ protein was higher in an adenocarcinoma cell line (TE-7) than in a squamous cell carcinoma cell line (TE-1). The authors concluded that PPARγ inhibition in human esophageal adenocarcinoma cell lines leads to induction of apoptosis, cell cycle arrest, and reduced ornithine decarboxylase activity (20). In the light of our findings, these data are misleading because TE-7 is not an adenocarcinoma cell line.

Based on the results of xenografting, STR profiling, mutation analyses, and array-CGH, we conclude that the currently used TE-7 cell line is not an esophageal adenocarcinoma but a squamous cell carcinoma cell line. Furthermore, cell line TE-7 shares the same genotype as TE-2, TE-3, TE-12, and TE-13. These five TE cell lines should be regarded as one single squamous cell carcinoma cell line. Our study emphasizes that researchers should be careful in the way they establish and use cell lines because a mistaken identity of a cell line may lead to invalid conclusions. Therefore, we suggest that future established tumor cell lines must be genotyped by SNP or STR analysis and compared with the patient’s tumor and normal tissue genotype. This genotyping information must be made available for the cell line users so that regular checks on the identity can be done.

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References

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