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BRIEF COMMUNICATION

Verification and Unmasking of Widely Used Human Esophageal Adenocarcinoma Cell Lines

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For decades, hundreds of different human tumor type–specific cell lines have been used in experimental cancer research as models for their respective tumors. The veracity of experimental results for a specific tumor type relies on the correct derivation of the cell line. In a worldwide effort, we verified the authenticity of all available esophageal adenocarcinoma (EAC) cell lines. We proved that the frequently used cell lines SEG-1 and BIC-1 and the SK-GT-5 cell line are in fact cell lines from other tumor types. Experimental results based on these contaminated cell lines have led to ongoing clinical trials recruiting EAC patients, to more than 100 scientific publications, and to at least three National Institutes of Health cancer research grants and 11 US patents, which emphasizes the importance of our findings. Widespread use of contaminated cell lines threatens the development of treatment strategies for EAC.

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Cell lines derived from human cancers have been crucial to building our understanding of the molecular pathophysiology of cancer and its treatment. Of equal importance, they form an in vitro model system for rational drug discovery and development because they are easy to maintain and manipulate in vitro and in animal xenograft models. However, it has been estimated that up to one-third of all cell lines have an origin other than that supposed (1). Cross-contamination between cell lines and mislabeling of cultures lead to unrecognized cell line admixtures (1,2). In the past, the scientific community has recognized this problem, but decisive action has not been taken to date. Results based on experiments using contaminated cell lines might be translated to the clinic, forming the basis for clinical trials, and directly affecting the treatment of patients.

Model research on esophageal adenocarcinoma (EAC), which is the cancer type showing the steepest rise in incidence in the Western world over recent years (3), relies entirely on a relatively small set of established tumor cell lines. Appropriate animal models and familial cases for EAC are lacking (4). Cell lines are very useful to investigate molecular pathways that are involved in EAC tumorigenesis and to test experimental drugs on EAC cells in vitro and in vivo. Despite intensive efforts to culture EAC cells in vitro, only 14 permanent cell lines have been established: SEG-1, BIC-1, and FLO-1 (5); SK-GT-4, SK-GT-5, and BE-3 (6); KYAE-1 (7); OE19 and OE33 (8); JH-EsoAd1 (9); OACP4C and OACM5.1 (10); and two newly established cell lines ESO26 and ESO51 (by Grupo de Estudos de Esófago de Barrett do IPOLFG, Lisbon, Portugal). In collaboration with the primary investigators who established the cell lines, the original EAC tissues for 13 of the 14 cell lines were traced in pathology archives and made available for study (anonymously): The original tissue for cell line BE-3 (6) was not found. The availability of the primary tissues made it possible to authenticate these EAC cell lines by comparing the genotype of the cell line with the genotypes of patient's normal and tumor tissue (see Supplementary Materials and Methods, available online, for detailed methods). Genotyping was performed by short tandem repeat profiling using the polymerase chain reaction–based Powerplex 16 System (Promega, Madison, WI) (1). To further verify the authenticity of the cell lines, *TP53* mutation analysis was performed (11). All exons and intron–exon boundaries of the *TP53* gene were sequenced in all the EAC cell lines (Asper Biotech Ltd, Tartu, Estonia). The *TP53* (GenBank accession number AF307851.1) mutations identified in the cell lines were then investigated in the original tumor tissues from which the cell lines had been derived. Ten of the 13 cell lines unambiguously had the same genotype and harbored the same *TP53* mutation(s) as the original tissues, proving their correct derivation (Table 1 and Supplementary Table 1, available online). The most frequently used EAC cell lines SEG-1 and BIC-1 and the SK-GT-5 cell line had genotypes different from the original tissue, of which the cell line was stated. Comparison of the genotypes of SEG-1, BIC-1, and SK-GT-5 with genotypes available from databases (http://www.lgcstandards-atcc.org/ATCCCulturesandProducts/CellBiology/STRProfileDatabase/tabid/986/Default.aspx) revealed that SEG-1 is lung carcinoma (large cell lung cancer) cell line H460 (ATCC_HTB-177) and BIC-1 is colorectal adenocarcinoma cell line SW620 (ATCC_CCL-227). The genotype and *TP53* mutation of the SK-GT-5 cell line matched with the tissue from which the cell line SK-GT-2 was derived, indicating that cell line SK-GT-5 is actually the gastric fundus carcinoma cell line SK-GT-2 (Supplementary Table 2, available online). In independent experiments, the researcher who established cell lines SEG-1 and BIC-1 (D. G. Beer) confirmed our results using the earliest passages of these cell lines. Clearly, contamination occurred early during establishment of the cell lines, and all of the cultures that were distributed subsequently to different laboratories were contaminated.

CONTEXT AND CAVEATS

Prior knowledge

Human tumor cell lines are commonly used in basic cancer research as preclinical models of human cancer. Research on esophageal adenocarcinoma relies heavily on these cell lines because of the limited availability of patient samples and animal models.

Study design

In collaboration with the primary investigators who established the cell lines, the authenticity of all currently available esophageal adenocarcinoma cell lines were examined using data from pathology archives and genotyping assays.

Contribution

Three commonly used cell lines were identified as being contaminated and were confirmed as being tumor types other than esophageal adenocarcinoma. Two of these cell lines have been used in 11 US patents and in more than 100 published studies, which have led to clinical trials of esophageal adenocarcinoma patients. The 10 cell lines whose authenticity was verified will be placed in public repositories to promote future research.

Implications

The development of treatments for esophageal adenocarcinoma may be negatively affected by the widespread use of these contaminated cell lines.

Limitations

It was not possible to include in this analysis studies that have not been published that may also be using the contaminated cell lines or that were based on results from studies using the contaminated cell lines.

From the Editors

We obtained the earliest available passage of cell line SK-GT-5 (1993) from D. S. Schrump (on referral by N. K. Altorki). This sample matched tissue from which cell line SK-GT-2 was derived, indicating that cross-contamination had occurred at the site of origin or during the early interinstitutional exchange of cell line SK-GT-5.

After hundreds, perhaps thousands, of culture passages of the 10 verified EAC cell lines, it could be questioned how representative these cell lines still are as models for their original EAC tumors. In the 10 verified EAC cell lines, 11 *TP53* mutations

Table 1. Short tandem repeat profiles (number of repeats at each locus is indicated) of 14 established esophageal adenocarcinoma cell lines*

	Short tandem repeat locus														
Cell line	**Penta E**	**D18S51**	**D21S11**	**TH01**	**D3S1358**	**FGA**	**TPOX**	**D8S1179**	**vWA**	**Penta D**	**CSF1PO**	**D16S539**	**D7S820**	**D13S317**	**D5S818**	**Amg**
SEG-1	5	13, 15	30	9.3	15, 18	21, 23	8	12	17	11, 13	11, 12	9	9, 12	13	9, 10	X, Y
BIC-1	10	13	30, 30.2	8	15, 16	24	11	13	16	9, 15	13, 14	9, 13	8, 9	12	13	X
FLO-1	5, 17	14, 16	30, 32.2	6	15	21	9, 11	13	16	11, 12	11	12, 13	8	11	12, 14	X
KYAE-1	5, 8	14, 15	29, 31	6	15, 16	18	8	13, 14	14	9, 10	11	11	11, 12	11, 12	10, 13	X
OE19	5, 8	12, 13	30	8, 9	15, 18	23, 26	8	13, 15	16, 17, 18	9	11, 13	12, 13	8	9, 11	11, 14	X
OE33	12, 18	12	29, 31.2	7, 8	18	23	8, 11	10, 11, 12	17	9, 11	10, 11	12	9, 10	14	11	X
OACM5.1	7, 14	16	28, 31	6, 9.3	16, 17	22	8	13, 14	19, 20	10	10, 13	10, 11	8	11, 12	12	X
OACP4C	20	12, 13	30	9	18	20	11	13	16	12	11	12	9, 11	12	9	X
SK-GT-4	7	14	31.2	6, 9.3	17	22, 23	8, 10	13	17, 19	9	11, 15	11, 12, 13	7, 11	9, 10	12	X
SK-GT-5	15, 17	14, 15	32.2	8, 9	15	26	9, 12	15	15, 18	9	10	11, 12	9, 10	12, 13	10	X
BE-3	10, 16	17	30.2	9	16, 17	22	8, 10	12, 14	18	13	11	12	8, 11	11, 12	11, 13	X
JH-EsoAd1	11	12	30	6, 7	16	24	8, 9	10	18, 19	14	10	10, 12	10, 12	11	11	X
ESO26	12, 14	17	30	9	15	21	7, 9	13, 14	14, 18	10	9, 10	9	11	9, 11	12	X, Y
ESO51	17	17, 20	30, 33.2	9	15, 16	21, 22	8	10, 11, 12	14, 15	9, 13	10	13	8	11	11	X

* The profile of cell line SEG-1 is identical to large cell lung carcinoma cell line H460. The profile of cell line BIC-1 is identical to colon adenocarcinoma cell line SW620. The profile of cell line SK-GT-5 is identical to gastric adenocarcinoma cell line SK-GT-2. Amg = Amelogenin.

were identified, and all were found to be present in the respective original tumor tissues (Supplementary Table 3, available online), indicating consistency of the *TP53* mutations during decades of in vitro propagation of the cell lines. Furthermore, xenografts obtained from nine of the 10 verified EAC cell lines all showed a phenotype identical to the original EAC tumors, demonstrating that the cellular features and architecture of the tumor are preserved even after long term in vitro culture (Figure 1).

The impact of our findings is illustrated by two clinical trials that are currently recruiting EAC patients based on experimental results using the contaminated cell lines SEG-1 and BIC-1. The first trial (http://clinicaltrials.gov/ct2/show/NCT00619242) investigates the effect of sorafenib (BAY 43-9006), a potent competitive small-molecule multikinase inhibitor of the Raf/MAPK/ERK pathway, on Barrett-related EAC. Several studies suggested that exposure of cell line SEG-1 to acid increased proliferation and decreased apoptosis by activating the Raf/MAPK/ERK pathway (12–14). In addition, treatment of SEG-1 cells with Raf/MAPK/ERK inhibitors resulted in pronounced antiproliferative effects (15–18). In this study, we have proved that cell line SEG-1 does not represent EAC but large cell lung carcinoma. This means that there is scant scientific evidence for activation of the Raf/MAPK/ERK pathway by acid or bile exposure in Barrett-related adenocarcinoma. The use of sorafenib in patients with Barrett-related EAC and the recruitment of patients for this clinical trial should therefore be reconsidered.

Another potential target for therapy in EAC, which is based mainly on research with contaminated cell lines SEG-1 and BIC-1, is telomerase (19,20). In a recent study, investigators demonstrated that treatment of SEG-1 xenografts with a specific telomerase inhibitor, GRN163L, led to loss of telomerase activity, reduction of telomere length, and inhibition of cell growth through induction of both senescence and apoptosis (21). Currently, EAC patients (among patients with other cancer types) are being recruited within a phase I clinical trial to study the effects of this telomerase inhibitor (http://clinicaltrials.gov/ct2/show/NCT00310895?term=GRN163L&rank=2). Our findings suggest that there is little scientific evidence for treatment of EAC patients with telomerase inhibitor GRN163L.

We have identified more than 100 scientific publications in which the contaminated cell lines SEG-1, BIC-1, or SK-GT-5 were used (Supplementary Tables 4 and 5, available online). Almost half of these reports were based solely on the use of cell lines not representative for EAC and should therefore be reevaluated because these cell lines in reality represent large cell lung carcinoma (SEG-1), colon adenocarcinoma (BIC-1), or gastric fundus adenocarcinoma (SK-GT-5). In addition, at least three National Institutes of Health grants have been assigned to esophageal cancer research projects (http://crisp.cit.nih.gov/), and 11 US patents (http://www.uspto.gov/) have been granted based on the use of cell lines SEG-1 and BIC-1. Following the lead of Walter Nelson-Rees, the first person who urged scientists to stop using contaminated cell lines (22,23), this report is a call for all scientists to authenticate their cell lines. Recent advances in DNA-profiling techniques make it possible to genotype cell lines simply and cheaply. The use of verified cell lines is a shared responsibility of scientists, editorial boards of scientific journals, and clinical and basic cancer research funding agencies.

In summary, cell lines SEG-1, BIC-1, and SK-GT-5 are not EAC cell lines but large cell lung cancer cell line H460, colorectal adenocarcinoma cell line SW620, and gastric fundus carcinoma cell line SK-GT-2, respectively. Cell lines FLO-1,

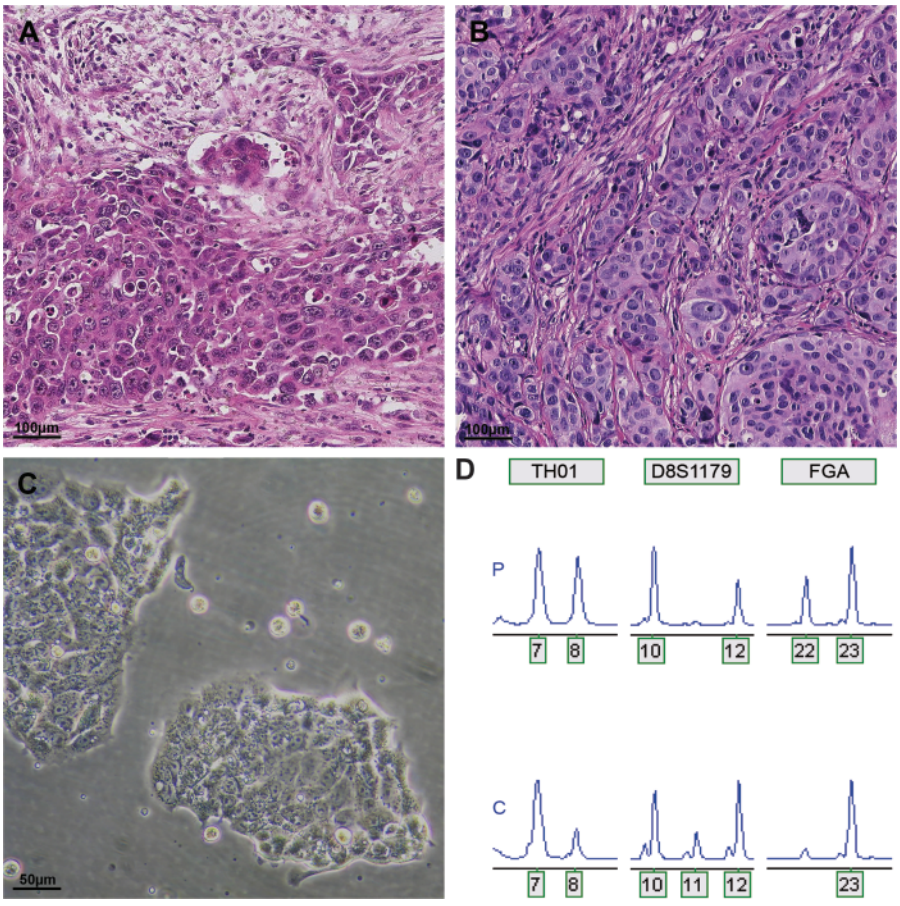

Figure 1. Authentication of human esophageal adenocarcinoma cell line OE33. **A and B**) Hematoxylin- and eosin-stained sections of the original tissue and xenograft of OE33 showing a poorly differentiated adenocarcinoma. **C**) In vitro growth pattern of cell line OE33. **D**) Short tandem repeat profile of the primary normal tissue (P) and cell line OE33 (C) indicating correct derivation of the cell line. Short-term repeat loci are indicated in **boxes** above electropherogram; the **number of repeat units** is indicated below the peaks. Note: loss of heterozygosity in the cell line at loci TH01 and FGA. The additional allele (11 repeat units) of D8S1179 observed in cell line OE33 is a known phenomenon and is probably due to somatic mutation or localized chromosomal rearrangements at this heterozygous locus.

KYAE-1, SK-GT-4, OE19, OE33, JH-EsoAd1, OACP4C, OACM5.1, ESO26, and ESO51 are derived from human EACs. All of these 10 verified EAC cell lines, together with their genotyping information, will be deposited in publicly available cell line repositories in the United States (http://www.lgcpromochem-atcc.com), Europe (www.ecacc.org.uk), and Japan (www.brc.riken.jp) to promote and facilitate future solid research on EAC.

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