TSU-Pr1 and JCA-1 Cells Are Derivatives of T24 Bladder Carcinoma Cells and Are Not of Prostatic Origin

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Abstract

We have shown previously that the putative prostate carcinoma cell lines TSU-Pr1 and JCA-1 share a common origin. The observation that these cell lines have p53 and Ha-ras mutations identical to those in bladder carcinoma cell line T24 prompted us to investigate their possible interrelations. We used cytogenetics and DNA profiling to compare the genetic backgrounds of the three cell lines. At least 12 structural chromosomal abnormalities are shared between T24, TSU-Pr1, and JCA-1 cells. DNA profiles were identical for all three cell lines. These results clearly indicate that the cell lines TSU-Pr1 and JCA-1 are not of prostatic origin but are derivatives of the bladder carcinoma cell line T24. TSU-Pr1 and, to a lesser extent, JCA-1 are frequently used as models in prostate cancer research, and numerous publications have appeared based on these lines. Several other T24 cross-contaminants have been identified in the past, and some of these, such as ECV304, continue to be used under the wrong identity. Our findings highlight the insidious problem that can occur when information regarding cross-contamination does not reach individual researchers and/or the importance of the problem is not fully acknowledged.

Introduction

Ever since the first mammalian cell lines were established, problems with cross-contamination have seriously compromised research. The problems discovered in the 1950s were largely due to interspecies contamination (1). In the 1960s, Stanley Gartler made the shocking discovery that most (if not all) of the putative unique human cell lines available at that time were actually derivatives of the HeLa cell line (2). Although, Gartler’s findings increased awareness, in the 1970s the sources and cell repositories, whereas many cell lines in use in these researchers only investigated cell lines obtained from original scientists, whereas some cell lines in use in individual laboratories are obtained indirectly. Moreover, they did not have access to many of the older cell lines for comparison. Despite renewed attention to this problem and improved electronic access to the pertinent data, a large portion of the scientific community is still apparently unaware of or indifferent to this problem. Not only does it sometimes take decades before misidentified cell lines are unmasked, but their inappropriate use continues because this information does not reach the individual scientist or is somehow not perceived as being important.

We have previously reported on the common origin of several prostate carcinoma cell lines (7, 8). In van Bokhoven et al. (7), TSU-Pr1 and JCA-1 cells were shown to have originated from the same individual. TSU-Pr1 and JCA-1 are frequently used as prostatic carcinoma models. TSU-Pr1 was originally described in 1987 as having been derived from a prostate carcinoma lymph node metastasis (9), and JCA-1 was described in 1990 as having been derived from a primary prostate carcinoma (10). Identical p53 and Ha-ras mutations in these two cell lines and in a third cell line, T24, prompted us to investigate the possibility that all three are related. The T24 cell line was established in 1970 from a urinary bladder carcinoma (11). We used cytogenetics and DNA profiling to compare the chromosomal and genetic background of T24 cells with those of TSU-Pr1 and JCA-1 cells. We discovered that TSU-Pr1 and JCA-1 cells, originally believed to be of prostatic origin, are actually derivatives of the bladder carcinoma cell line T24.

Materials and Methods

Cells and Cell Culture. TSU-Pr1 cells were a kind gift from Dr J. Isaacs (Johns Hopkins University, Baltimore, MD), JCA-1 cells were a kind gift from Dr. J. Chiao (New York Medical College, Valhalla, NY), and T24 cells were purchased from the American Type Culture Collection (HBT-4; Manassas, VA). All cell lines were grown in RPMI 1640 supplemented with 10% fetal bovine serum. Cultures were maintained in a humidified atmosphere of 5% CO2 in air at 37°C.

Cytogenetic Analysis. Cells were blocked in metaphase with Colcemid (0.05 μg/ml) for 1–2 h before hypotonic swelling in a 4:1 mixture of 0.075 M KCl and 1% sodium citrate. Fixation was done using a 3:1 mixture of methanol and glacial acetic acid. Slides were prepared and G-banded according to a standard protocol (7, 8), except that chromosomes were stained with Leishman’s stain. Metaphase chromosomes were digitally imaged and karyotyped with a CytoVysion System (Applied Imaging, Santa Clara, CA). M-FISH4 was performed on TSU-Pr1 and JCA-1 cells as described previously (7, 8). The T24 cell line was examined by SKY. The SKY probe mixture and hybridization reagents were prepared by Applied Spectral Imaging (Carlsbad, CA). A combinatorial labeling of five fluoros, FITC, Texas red, rhodamine, Cy5, and Cy5.5, was used to generate the 24 colors. Image acquisition was performed using a SD200 Spectracube (Applied Spectral Imaging) coupled to an Olympus BX60 epifluorescence microscope and a custom optical filter (SKY-1; Chroma Technology, Brattleboro, VT). Analysis was performed using SKYView v1.6 software (Applied Spectral Imaging). In both M-FISH and SKY assays, at least 10 metaphase spreads were completely karyotyped for each cell line, and abnormalities were listed as clonal when present in at least two cells. Karyotypes were interpreted according to the International System for Human Cytogenetics.

4 The abbreviation used is: M-FISH, multiplex fluorescence in situ hybridization; SKY, spectral karyotyping.

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Cytogenetics Nomenclature guidelines (12). The positions of the chromosome breakpoints were assigned, when possible, based on inverted 4',6-diamidino-2-phenylindole images and/or on the G-banding results obtained previously.

**Mutation Analysis.** The exon 5–9 region of p53 was amplified by reverse transcription-PCR with primers 5'-CAGCATACATGACGAGGTGTG-3' and 5'-GGAGAGAGGCGTGGTGTGTTG-3'. The area spanning codon 12 of Ha-ras was amplified from genomic DNA using primers 5'-CCTGGGGCTTGCTGAGCAG-3' and 5'-ACCATGCGGGACAGAAGG-3'. The cycling conditions were described previously (7). The primers used for PCR were also used for direct sequencing of PCR products.

**DNA Profiling Analyses.** The AmpF/STR Profiler Plus kit (Applied Biosystems, Foster City, CA) was used for DNA profiling analysis according to the manufacturer’s instructions. This PCR-based kit uses primers labeled with different fluorophores (F-AFM, JOE, and NED) to amplify nine Short Tandem Repeat markers and a gender marker in a single reaction tube. The markers (and their chromosomal locations) included were D3S1358 (3p), VWA (12p12-pter), FGA (4q28), D8S1179 (8), D21S11 (21), D18S51 (18q21.3), D5S818 (5q12–31), D13S317 (13q22–31), D7S820 (7q), and amelogenin (X, Y). The amplicons were analyzed on an ABI 377 DNA Automated Sequencer using GeneScan version 3.1 (Applied Biosystems).

### Results

Upon arrival in our laboratory, the karyotype of each cell line was determined using G-banding and compared with previously published karyotypes to confirm their authenticity. Subsequently, multicolor karyotyping (M-FISH in TSU-Pr1 and JCA-1 cells and SKY in T24 cells) was performed to permit a more detailed comparison of the different cell lines. T24 was only introduced into our laboratory after our studies on TSU-Pr1 and JCA-1 cells had been completed. The karyotypes of the TSU-Pr1 and JCA-1 cell lines were found to be similar to those in the original published descriptions (9, 10). Therefore, we used the published karyotypes of Hastings and Franks (13) and Gildea et al. (14) as references for comparison. Considering our results, the publications taken as references, and the recently published spectral karyotypes by Pan et al. (15), we identified a total of 12 shared structural abnormalities among these three lines, which are listed in Table 1. Some of the shared abnormalities were not found consistently in a given cell line either because intercellular heterogeneity remained undetected due to the small numbers of cells analyzed or because technical ambiguity existed in previous publications. In addition, a number of rearrangements received distinct designations in individual publications. For instance, the der(2;20)(q10;q10) that we found in JCA-1 strikingly resembled the chromosome designated 2p− in JCA-1 by Muraki et al. (10), 20q+ in TSU-Pr1 by Izumi et al. (9), and the der(2;20)(q11;p11) in TSU-Pr1 by Pan et al. (15).

We reported previously that TSU-Pr1 and JCA-1 shared a total of five similar and three sequential abnormalities with each other (7). In this current, more inclusive analysis, there were seven abnormalities that were shared among all three cell lines. Furthermore, TSU-Pr1 and JCA-1 were each found to share, respectively, three additional abnormalities and one additional abnormality with T24. One abnormality was shared only by TSU-Pr1 and JCA-1. Overall, T24 shared 11 abnormalities with TSU-Pr1 and/or JCA-1 (Table 1); 7 of these are illustrated in Fig. 1.

The presence of the previously described mutation in p53 [codon 125 TAC→TAG, tyrosine→stop (16, 17)] in T24 cells was confirmed using reverse transcription-PCR, and the Ha-ras mutation [codon 12 GGC→GTC, glycine→valine (18)] was confirmed by PCR using genomic DNA as input. We have described previously that these mutations occur in both TSU-Pr1 and JCA-1 cells (7).

DNA profiling using genomic DNA from the three cell lines was performed. As we described earlier, the DNA profile of JCA-1 cells is illustrated in Fig. 1.
identical to the profile of TSU-Pr1 cells (7). Fig. 2 shows that, in addition, these DNA profiles are identical to that of T24 cells.

Discussion

This study identifies the cell lines TSU-Pr1 and JCA-1, which hitherto were believed to be of prostatic origin, as being derivatives of the bladder carcinoma cell line T24. Previously, we suggested that this might be the case based on the identical p53 and Ha-ras mutations shared by these three cell lines (7). Using classical cytogenetic analysis, similarities among these lines have remained undetected. The recently developed multicolor karyotyping techniques have significantly improved the ability to identify a number of similarities that argue strongly for the common origin of these cell lines. Using the chromosomal designations generated by multicolor karyotyping as a guide, we were also able to show that most of these similarities were evident in previously published karyotypes (Table 1). We did not detect a Y chromosome in TSU-Pr1 or JCA-1 cells. However, the original descriptions of these cell lines both mention the presence of a Y chromosome (9, 10). Ironically, this fact was used as proof that these cells were unrelated to HeLa cells. We believe that this chromosome was misidentified in previous publications because, using M-FISH and SKY analysis, we reclassified Y-like chromosomes as chromosome 15 derivatives (Table 1). T24 cells are of female origin and do not contain a Y chromosome (11). Our cytogenetic results were confirmed by the DNA analysis because no Y allele for the gender marker in the DNA profiling set, amelogenin, could be detected in any of the three cell lines (Fig. 2). The complete concordance between the DNA profiling results for all three cell lines provides unequivocal proof that they are all derived from the same individual.

In our previous report (7), we assumed that JCA-1 cells had

![Fig. 1. Shared chromosomal structural abnormalities among T24, TSU-Pr1, and JCA-1 cells. Analysis of T24 cells was performed using SKY, and analyses of TSU-Pr1 and JCA-1 cells were performed using M-FISH. Complete chromosome designations are presented in Table 1.](image)

![Fig. 2. DNA profiling analyses of T24 and TSU-Pr1 cells, allele of ladder.](image)
originated from TSU-Pr1 cells. In that scenario, it was difficult to explain the absence of a normal chromosome 15 in TSU-Pr1 cells and its presence in JCA-1 cells. This controversy no longer exists because it is now apparent that both cell lines originated independently from T24 cells, which contain apparently normal copies of chromosome 15. In the original description, prostate-specific antigen expression was identified in JCA-1 cell xenografts (10). However, this feature was rapidly lost in vitro. The most plausible explanation for these findings is that the xenografts still contained prostate carcinoma cells that were rapidly overgrown by T24 cells in vitro. No mention was made of prostate-specific antigen production by TSU-Pr1 cells in the original description of this cell line (9).

The $p53$ mutation that we detected in codon 126 (TAC→TAG) in TSU-Pr1, JCA-1, and T24 cells is uncommon. In addition, it has only been reported in a squamous cell carcinoma of the head and neck, in the cell line (HOC605) derived from this tumor, and in the bladder carcinoma cell line BT-1 (11). Comparison of the published BT-1 (19) karyotype with our T24 karyotype reveals several possible similarities indicating that BT-1 cells might also be a product of T24 contamination. No karyotype has been published for HOC605 cells.

T24 is recognized to have been the source of contamination in numerous cases. Most of these (EJ/MGH-U1, MGH-U2, Hu456, Hu549, Hu961T, HCV29Tmv, and Hu609Tmv) were already accredited to a bladder origin and subsequently identified as cross-contaminants identified in our study (30–22). Additional cell lines from other organ sites (ECV304, HAG, RAMAK-1, and GHE) have also been identified as cross-contaminants from T24 (6). ECV304, the most frequently used cell line in endothelial research, was first described in 1990 as a spontaneously transformed and immortalized human endothelial cell line (23). However, in 1999, it was found to be the result of contamination by T24 cells (6). The Medline database currently contains more than 250 publication entries using this cell line, more than 60 of which have appeared after the initial report indicating that ECV304 cells were actually T24 bladder carcinoma cells. Less than half of these reports correctly describe ECV304 as a derivative of T24, whereas the rest of the reports do not mention this fact. Whereas for some research it might not be critical whether the cell line is of bladder or endothelial origin, scientific progress hinges on accurate information. Interestingly, some reports mention the fact that the ECV304 cell line is a subclone of T24 cells but still use the ECV304 cell line as an endothelial model. Although T24 might have some endothelial characteristics, it is probably not the best endothelial model available (24). There are even older examples of cell lines that have been identified as cross-contaminants and are still in use under the wrong identity. For example, in 1967, KB cells, which had been believed to be derived from an epidermoid carcinoma, were identified as HeLa cross-contaminants by Gartler (2), a finding subsequently confirmed by others (3, 4). Still, hundreds of publications have since appeared (some dated as recently as 2001) in which KB cells have been used as an epidermoid carcinoma cell line.

TSU-Pr1 cells and, to a lesser extent, JCA-1 cells are frequently used as prostate carcinoma models. A recent Medline search identified more than 150 reports using these cells. Even our own laboratories have used them until recently as prostate carcinoma cells. The fact that bladder and prostate cells have the same embryological origin may make some findings obtained using bladder carcinoma cells applicable to prostatic adenocarcinoma cells. However, in other studies in which TSU-Pr1 or JCA-1 cells were incorrectly assumed to be of prostate carcinoma origin, conclusions regarding prostate carcinogenesis could be highly questionable.

This report accentuates the need for a definitive solution to the cell culture cross-contamination problem and the associated difficulties in dissemination of this information. Although reports in the past have suggested suitable solutions for these problems (5, 25, 26), they have never been widely implemented. We concur with Stacey et al. (27), who stated in a recent letter to Nature that this important problem requires urgent attention. DNA profiling has proven to be an easy, accurate, and economical method for cell line identification (6, 7, 26). Development of an online reference database for cell line characteristics, including DNA profiles, would make it easier for individual researchers to check the authenticity of their cells. Publications on new cell lines should always include a DNA profile of the cell line next to a profile of source DNA. The need to collect this information immediately, as well as archive the original DNA, is emphasized by our inability to obtain tissue from the patients reported to be the origins of TSU-Pr1 and JCA-1. Furthermore, it would be highly preferable for new cell lines to be submitted to culture collections like the American Type Culture Collection and the German Collection of Microorganisms and Cell Cultures (DSMZ) for independent authentication and further distribution to the scientific community. Cross-contaminants must be clearly identified in catalogues of culture collections to prevent their incorrect use. For most research, it would be preferable to use the original cell line instead of the cross-contaminant. However, occasionally, the subline may have acquired certain phenotypic characteristics that make it interesting. In these cases, it should be properly mentioned that a cross-contaminant has been used, and the cell and tissue of origin should be clearly stated. Stacey et al. (27) suggested the inclusion of the name of the cell line from which it originated, in addition to the “official” cell name. For the cross-contaminants identified in our study, we suggest the following designations: TSU-Pr1(‘T24’) and JCA-1(‘T24′) bladder carcinoma cells.

Applying these guidelines might eventually solve the problem; however, past experience indicates that the chances of that occurring are extremely low. Meanwhile, researchers, reviewers, and funding agencies need to become fully aware of this problem and the impact it could have on research. Without action, research findings obtained using cell lines under false identities will continue to pollute the scientific literature. The estimated 20–30% cross-contaminant cell lines in use are undoubtedly responsible for numerous misleading publications. This misinformation can potentially have a very high cost in terms of invalid hypotheses and paradigms, misspent effort, and protracted development of patient treatments.

In summary, the TSU-Pr1 and JCA-1 cell lines appear to be derivatives of the T24 bladder carcinoma cell line. Although each has properties that could make it useful in tumor biology research, it is clear that they can no longer be used as models for the study of prostate cancer.

References

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