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Kaposi's sarcoma-derived cell line SLK is not of endothelial origin, but is a contaminant from a known renal carcinoma cell line

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Kaposi's sarcoma (KS) is an endothelial cell-derived tumor. Investigations of the molecular mechanisms of KS pathogenesis and the identification of drugs for treatment of KS depend critically on valid cell-culture models. Two major immortalized cell lines are available for KS research. Recently, the KS cell line KS Y-1 has been shown to be cross-contaminated with the T24 urinary bladder cancer cell line (ATCC HTB-4). Here, we show by short tandem repeat profiling that the second KS cell line, SLK, is indistinguishable from the clear-cell renal-cell carcinoma cell line Caki-1. Immunocytochemical detection of cytokeratin expression confirmed the epithelial-cell origin of SLK cells. Our findings indicate that SLK cells are not of endothelial origin and should not be used in future studies as a model for KS-derived endothelial tumor cells. We suggest that in the future, more attention needs to be paid to the authenticity of cells in lines derived from human tissues.

Kaposi's sarcoma (KS) is a multifocal tumor that appears in four epidemiologically distinct forms: AIDS-associated KS, iatrogenic KS, endemic KS and African KS. The high-clinical impact of the disease is based on the facts that AIDS–KS was among the first clinical markers of the outbreak of the pandemic AIDS and that African KS is among the most frequent cancers in Central Africa. The tumor cells of KS are of endothelial-cell lineage with remaining uncertainty whether they may be derived from blood vessel or lymphatic vessel endothelial cells.[1] Because of their morphologic presentation in histological sections of KS tissues, these cells are called KS spindle cells (KSC). KS-associated herpesvirus (KSHV), also known as human herpesvirus-8, is the etiologic agent of KS.[2] In KS tumors, almost 80% of the KSC are infected with KSHV.[3] Recent studies indicate that KSHV infection induces a cellular reprogramming of blood vessel and lymphatic vessel endothelial cells toward the gene expression profile of the other lineage, respectively.[4,5]

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Cell lines in KS research

Investigations of molecular mechanisms of KS pathogenesis and the identification of drugs that may be effective in the treatment of KS patients are dependent on the availability of valid cell-culture model systems. Accordingly, several groups have tried to establish cultures of the KSC. In most cases, the isolated cells ceased growth in culture between passages 10–30 as a typical feature of primary cultures.[6–8] However, three groups succeeded in the isolation of immortal cells from KS patients and establishing continuously growing cell cultures. The respective cell lines were called KS Y-1, SLK and KS^{imm}.[9–11] Among these, KS Y-1 and SLK cells are the most broadly distributed and widely used.

KS Y-1 cells were derived from mononuclear cells isolated from 2.5 L of plural effusion of an AIDS patient with KS after selective removal of lymphocytes, monocytes/macrophages and fibroblasts by cytotoxicity methods.[9] The phenotype of these cells has initially been described as similar to that of endothelial cells, and despite being negative for KSHV,[12] this cell line was used for numerous studies. The most prominent findings heralded the use of human chorionic gonadotropin (hCG) as a putative KS inhibiting drug, based on xenotransplant experiments of KS Y-1 cells and reduced tumor growth in pregnant mice.[13] These findings resulted in the initiation of clinical studies on hCG in KS therapy, which after some initial conflicts, was commonly found to be astronomically expensive and unfortunately ineffective in KS therapy.[14] The reason for this failure became apparent when the KS Y-1

Short Report

What's new?
The KS Y1 and SLK cell lines are widely used for the study of Kaposi's sarcoma. KS Y1 cells, however, were discovered by ATCC to have a similar short tandem repeat (STR) profile as T24 urinary bladder cancer cells. Investigation of SLK cells here reveals that these cells also are contaminated, having an STR profile indistinguishable from that of Caki-1 renal cell carcinoma cells. The results indicate that SLK cells should not be used as model systems for Kaposi's sarcoma.

cells were deposited at the ATCC (ATCC CRL-11448) and were shown to exhibit a similar short tandem repeat (STR) profile as the T24 urinary bladder cancer cell line (ATCC HTB-4) [http://atcc.custhelp.com/app/answers/detail/a_id/1061/~/ksy-1-(atcc-crl-11448)].

The loss of KS Y-1 cells increased the importance of the SLK cell line. SLK cells were isolated in Israel circa 1990 from a tumor biopsy from the oral mucosa of an iatrogenically immunosuppressed HIV-1-negative man.[10,15] The cells were originally described as epithelial-like, and viral forms were reported in the cells.[15] Subsequently, the cells were sent to a laboratory in the United States (Dr. J. A. Levy), which reported in 1994 that no evidence of a virus could be detected, but based on immunocytochemical and electron microscopic examinations, it was suggested that these cells are of endothelial cell nature.[10] Specifically, the cells were found positive for endothelial cell-associated markers such as von Willebrand factor, EN4, ulex europaeus lectin binding and the urokinase receptor.[10] Moreover, electron microscopically Weibel–Palade bodies, which are storage granules characteristically present in blood vessel endothelial cells, were detected.[10] Although SLK cells did not contain KSHV, they were found to be tumorigenic in nude mice, and the resulting tumors were said to be well vascularized and reminiscent of KS.[10]

Around 2002, one of us (Dr. D. Ganem) obtained an aliquot of SLK from that lab and found that they were latently infectable with KSHV *in vitro* at high efficiency. Subsequently, these SLK cells were engineered to express a doxycycline (DOX)-inducible RTA gene.[16] RTA (replication and transcription activator) is the viral transcription factor whose expression governs the switch from latency to lytic replication in KSHV. The stably transfected cells were called iSLK cells, and a subline derived by latent infection with a recombinant KSHV [rKSHV.219[17]] is called iSLK.219 cells. The two iSLK cell lines were found very useful in the field, as they display strict control of latency. That is, they have very low levels of spontaneous lytic induction and are inducible with doxycycline at high efficiency. This has made them the line of choice for studying many aspects of viral gene regulation, and they have been widely disseminated for this purpose. Because of their presumed endothelial origin, it has also been suggested that they may be useful for studies of KS tumor biology.

Material and Methods

Cell culture

Caki-1, iSLK and iSLK.219 cells were cultivated in Dulbecco's modified Eagle medium (DMEM, PAA, Pasching, Austria) supplemented with 10% FCS, 2 mM L-glutamine at 37°C under 8.5% CO_2. The iSLK cells were cultivated in addition with 1 μg/ml puromycin and 250 μg/ml G418 and the iSLK.219 cells with 1 μg/ml puromycin, 250 μg/ml G418 and 250 μg/ml hygromycin. The SLK cells obtained through the NIH AIDS Research and Reagent Program were cultivated in RPMI 1640 medium (PAA) with 10% FCS and 2mM L-glutamine at 37°C under 5% CO_2. Primary human umbilical vein endothelial cells (HUVEC) were cultivated in endothelial growth medium 2 microvascular (EGM2-MV, Lonza, Cologne, Germany) at 37°C in a humidified atmosphere with 5% CO_2.

STR profiling

The short tandem repeat (STR) profiling technique was performed according to the guidelines published recently.[18,19] Briefly, SLK cells (obtained from the NIH biorepository), iSLK and iSLK.219 cells (obtained from Dr. Ganem) were expanded over three passages. Cells were frozen at 90% confluence during the exponential growth phase and sent for STR profiling analyses to the German Collection of Microorganisms and Cell Cultures [Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany]. STR DNA profiling was carried out using fluorescent PCR in combination with capillary electrophoresis as described previously.[20] Using different alternate colors, the PowerPlex® 1.2 system (Promega, Mannheim, Germany) was modified in order to run a two-color DNA profiling allowing the simultaneous single-tube amplification of eight polymorphic STR loci and Amelogenin for gender determination. STR loci of CSF1PO, TPOX, TH01, vWA and Amelogenin were amplified by primers labeled with the Beckman/Coulter dye D3 (green; Sigma-Aldrich, Munich, Germany), while the STR loci D16S539, D7S820, D13S317 and D5S818 were amplified using primers labeled with D2 (black). All the loci except the Amelogenin gene in this set are true tetranucleotide repeats. All primers are identical to the PowerPlex® 1.2 system except the fluorescent color. Data were analyzed with the CEQ 8000 software (Beckman-Coulter, Krefeld, Germany), which enables an automatic assignment of genotypes and automatic export of resulting numeric allele codes into the reference DNA database of the DSMZ.

Immunocytochemistry

Cells were seeded in their respective medium without selection antibiotics in chamber slides and cultivated at 37°C under 5% CO_2. The cells were grown in chamber slides, fixed

Table 1. STR analysis of SLK cells in comparison with Caki-1 reference genomes. [Color table can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

	STR allele																
Cell line	**D5S818**	**D5S818′**	**D13S317**	**D13S317′**	**D7S820**	**D7S820′**	**D16S539**	**D16S539′**	**vWA**	**vWA′**	**TH01**	**TH01′**	**TPOX**	**TPOX′**	**CSF1**	**CSF1′**	**Amel**	**Amel′**
Caki-1 (HTB-46^{TM})	11	12	11	12	8	12	12	12	15	17	6	8	8	11	10	11	x	x
Caki-1 (AMEC)	11	12	11	12	8	12	12	12	15	17	6	8	8	11	10	11	x	x
SLK (NIH)	11	12	11	12	8	12	12	12	15	17	6	8	8	11	10	11	x	x
iSLK	11	11	11	12	8	12	12	12	15	17	6	8	8	11	11	11	x	x
iSLK.219	11	11	11	12	8	12	12	12	15	17	6	8	8	11	11	11	x	x

Short tandem repeat (STR) profile patterns of Caki-1 cells ATCC reference (Caki-1 HTB-46^{TM}), Caki-1 cells available at the Division of Molecular and Experimental Surgery (Caki-1 AMEC), SLK cells from the NIH AIDS Research and Reference Reagent Program (SLK NIH) and iSLK and iSLK.219 from the Ganem Lab.[16] STR-analysis has been carried out with a PCR approach using nine different markers as described previously.[18]

with paraformaldehyde (for keratin staining) or ethanol (CD31 and von Willebrand factor staining) and subjected to immunocytochemistry as described elsewhere.[21] Cells were stained using the endothelial-cell antigen-directed antibodies anti-CD31 (Dako, Glostrup, Denmark, dilution 1 : 50), anti-von Willebrand factor (Dako, 1 : 50) and an epithelial cell-specific pan anticytokeratin AE1/AE3 antibody (DBS, Pleasanton, CA; 1 : 10).

Results

Authenticity of SLK cells

Here, we tested the authenticity of SLK cells using the STR-profiling technique according to the guidelines published recently.[18,19] The iSLK and iSLK.219 cells were examined using the recommended 9 STR loci to enable a scan of an international reference database of cell line STRs.[22] This revealed that both iSLK cells as well as iSLK.219 cells showed a profile similar to Caki-1 cells (ATCC HTB-46^{TM}; Table 1). The STR loci D5S818′ and CSF1 showed loss of one allele, respectively, a phenomenon known as loss of heterozygosity (LOH; Table 1, green box). LOH in cell cultures can be observed in the course of generating stable single clones of a transfected cell line due to selective outgrowth of a sideline within the cell population. The genetic identity of the cell lines presented in Table 1 is correct with high confidence, because the exclusion rate of the applied STR system is about 1 in 1.14×10^8. The iSLK cells used here were infectable with recombinant rKSHV.219, and the iSLK.219 cells were susceptible to the induction of lytic infection by treatment with DOX and produced infectious virus (data not shown). These facts confirmed that the cell lines were identical to those published.[16] Of note, iSLK and iSLK.219 cells exhibited an identical STR and LOH pattern, indicating that cross-contamination was already present in the originating culture, before the iSLK cells were generated.

The SLK cells from which iSLK were created have been obtained directly from Dr. Levy and subsequently have been in continuous culture in the Ganem Lab for over 10 years (2002–2012). Caki-1 cells have never been grown in that environment. Accordingly, we asked if SLK cells that had not been passaged there were already contaminated with Caki-1. The only independent US source of SLK of which we are aware is the NIH AIDS Research and Reagent Program, which received them from the laboratory of Dr. Levy, who had earlier obtained them from the line's originator, Dr. S. Leventon-Kriss. SLK cells were ordered from the NIH AIDS Research and Reagent Program (reagent no. 9402). STR analysis showed that these cells exhibited an identical profile to the reference STR profile of Caki-1 cells (ATCC HTP-46) and to Caki-1 cells, which were provided to the Erlangen group from colleagues in April 2012 [Caki-1 (AMEC; Table 1)]. These results demonstrated that, at a minimum, the SLK cell line must have been cross-contaminated before 2002. A search for rodent mitochondrial DNA sequences was

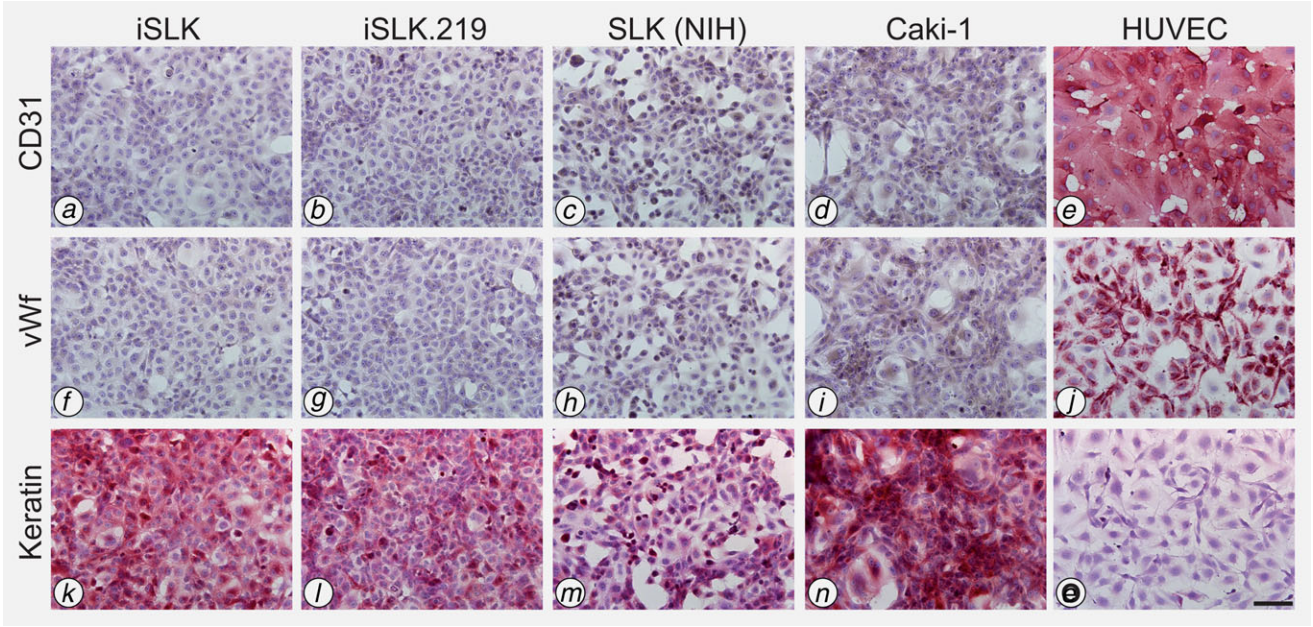

Figure 1. SLK cells exhibit epithelial cell-specific molecular marker staining. iSLK cells, iSLK.219 cells, SLK (NIH) cells, Caki-1 cells and HUVEC were stained with the endothelial cell antigen directed antibodies anti-CD31 (Dako, Glostrup, Denmark, dilution 1:50), anti-von Willebrand factor (Dako, 1:50) and with an epithelial cell-specific pan anticytokeratin AE1/AE3 antibody (DBS, Pleasanton, CA; 1:10). Cells were grown in chamber slides, fixed with paraformaldehyde (for keratin staining) or ethanol (CD31 and von Willebrand factor staining) and subjected to immunocytochemistry. Scale bar represents 100 μm and is representative for all figures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

negative, confirming that the cells were of solely human origin (data not shown).

To confirm that SLK cells are in fact of epithelial-cell lineage, cell-type-specific marker expression of SLK cells was analyzed by immunocytochemistry. This study confirmed that the iSLK cells, iSLK.219 cells and the parental SLK cells are identical with Caki-1 cells and different from endothelial cells, as they do not express the endothelial-cell markers CD31 (Figs. 1*a*–1*d*) and von Willebrand factor-related antigen (Figs. 1*f*–1*i*), but are positive for the epithelial cell-marker keratin (Figs. 1*k*–1*n*).

Discussion

Impact on KS and cell biological quality control

The major conclusion from these findings is that SLK cells, like KS Y-1 cells, have been cross-contaminated relatively early in their passage and cannot serve as an appropriate model system of Kaposi's sarcoma. All the SLK cell cultures that were available for us and which are representing the major stocks presently used in KS research were identical to Caki-1 cells, which are derived from a clear-cell renal-cell carcinoma. Investigators should refrain from using SLK cells in future studies as a model for KS-derived endothelial tumor cell biology or oncogenesis. However, the cells are fully permissive for the KSHV latent and lytic replicative cycles and retain their utility for the study of KSHV gene expression in nonendothelial contexts.

Of note, in initial studies, shortly, after isolation, SLK cells were found positive for endothelial cell-associated markers such as von Willebrand factor and ulex europaeus lectin binding.[10] However, a review of the available literature on the expression of endothelial-cell markers in KS lesions in the 1990s documented highly disconcordant findings with respect to the expression of these two markers.[23] This suggests that the staining methods with these markers were not at a reliable and reproducible standard at these times and should be treated with caution.

The pathogenesis of KS is still a matter of controversy, particularly whether KS is a true sarcoma or a reactive hyperplastic proliferation.[1,23,24] The isolation of three transformed cell lines from KS tumors supported the "sarcoma hypothesis." With the detection of cross-contamination of two of these cell lines, this argument has been clearly weakened. In this framework, it will be interesting to submit KS^{imm} cells to an authenticity test. However, none of the available cell-culture models was long-term infected with KSHV after isolation.[12,25]

Unfortunately, cross-contamination is not a rare event. Accordingly, the standards of characterization of cell-culture model systems should be significantly improved. This is of paramount importance for cell-culture models, which are used for translational research approaches, which subsequently may have impact on treatment of patients. An increasing number of journals require the addition of recent documents for cell authentication to paper submissions. As yet, these journals include the *International Journal of Cancer*, the *In Vitro—Animal Journal*, *Journals of Bentham Science* and the *American Association for Cancer Research*. It is

highly desirable that this may soon become a scientific standard procedure requested by all journals.

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Short Report