Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

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Position statement of the ZKBS on contamination of cell lines with Squirrel Monkey Retrovirus

Recently, two collaborating German laboratories reported extensive contamination of their cell cultures with Squirrel Monkey Retrovirus (SMRV) to the appropriate Federal State authorities, the Ministry for Agriculture, the Environment and Countryside of the State of Schleswig-Holstein and the Government presidium of Tübingen. The contamination was discovered by chance while analyzing expression profiles in recombinant cell lines. Examination of the state of the existing cell culture revealed that SMRV contamination had already been present for several years and was probably transmitted by spreading from one cell line to the other during laboratory procedures [1]. Further spread of this contamination is probable due to exchanging sample material and cell cultures with cooperating partners.

SMRV contamination in various vertebrate cell lines was described as early as 1982 [2]. In 1998, a human isolate of SMRV (SMRV-H) was detected in the Burkitt lymphoma cell line Namalwa, which was used for the commercial production of α-interferon. SMRV sequences could be detected in 39 out of 75 tested commercial interferon preparations from various producers in different countries [3].

The appropriate Federal State authorities requested the ZKBS to make a risk assessment of SMRV and recommendations for further handling of cell cultures in genetic engineering operations.

Assessment

Risk assessment of SMRV

SMRV is an endogenous type D retrovirus isolated from lung tissue of a squirrel monkey (*Saimiri sciureus*) in 1977, which according to the current classification of the International Committee on Taxonomy of Viruses was assigned to the family of beta retroviruses [4]. It shows homology to type A, type B and type C retroviruses [5]

Natural infections of other hosts have not been described. Based on the contamination of numerous vertebrate cell lines (ranging from human, dog, mink, old and new world monkeys) it apparently possesses a wide host tropism *in vitro* [5, 6].

There is no evidence of pathogenicity of SMRV for its natural host, non-human primates. Infections or symptoms in people treated with SMRV-contaminated preparations of interferon have not been described.

A low pathogenicity of SMRV cannot be excluded since it was shown in the cell line Namalwa that an incomplete SMRV-H proviral genome was inserted at the locus of the proto-oncogene c-myc [7]. Due to the wide host tropism and in individual cases the possibility of inducing activation of cellular oncogenes or altering the transcription activity of other regulatory genes through provirus insertion, a low risk potential for SMRV cannot be excluded.

In the Swiss directives (BUWAL) and the instruction leaflet B004 of the BG Chemie, SMRV is listed as an animal pathogenic organism belonging to risk group 2, and the ATCC recommends biological safety level 2.

The ZKBS similarly allocates SMRV as a donor and recipient organism in genetic engineering operations to **risk group 2** according to § 5 Paragraph 1 of the Genetic Engineering Safety Regulations (GenTSV) with reference to the criteria in Appendix I of the GenTSV.

Recommendations for handling cell cultures in genetic engineering operations

The ZKBS does not exclude the possibility of widespread contamination of cell lines with SMRV. Thus it recommends checking cell lines intended for use in genetic engineering operations for SMRV. Contaminated cell lines should be inactivated or kept under safety measures corresponding to biological safety level 2. In addition, the ZKBS points out that complementation of recombinant, replication-defective retroviruses in cell lines contaminated with SMRV cannot be excluded.

In addition, animals receiving cells or their supernatants are also possibly infected with SMRV and should be similarly checked or kept under conditions corresponding to biological safety level 2 safety measures.

It is requested that results on SMRV tested cell lines and about complementation of recombinant replication defective retroviruses should be reported to the ZKBS.

Notes:

You are referred to the general position statement of the ZKBS on frequently carried out genetic engineering operations based on the criteria of comparability: gene transfer using retroviral vectors, Ref. No. 6790-10-41, from June 1996.

The following sequences are suitable for PCR detection of SMRV:

env: Upper Primer: GGCGGACCCCAAGATGCTGTG

Lower Primer: TGGGCTAGGCTGGGGTTGGAGATA

gag: Upper Primer: TCAGAGCCCACCGAGCCTACCTAC

Lower Primer: CAGCGCAGCACGAGACAAGAAAA

References

1. A C van der Kuyl, R Mang, J T Dekker, and J Goudsmit. Complete nucleotide sequence of simian endogenous type D retrovirus with intact genome organization: evidence for ancestry to simian retrovirus and baboon endogenous virus. J Virol. 1997 May; 71(5): 3666–3676

2. Popovic M et al. Identification of the RPMI 8226 retrovirus and its dissemination as a significant contaminant of some widely used human and marmoset cell lines. Int J Cancer. 1982 Jul 15;30(1):93-9

3. Pienkowska M, Seth A. Detection of squirrel monkey retroviral sequences in interferon samples. J Hepatol. 1998 Mar;28(3):396-403.

4. RL Heberling, ST Barker, SS Kalter, GC Smith, and RJ Helmke. Oncornavirus: isolation from a squirrel monkey (Saimiri sciureus) lung culture. Science 21 January 1977:Vol. 195. no. 4275, pp. 289 – 292

5. Chiu Cindy N, Mitra R ; Chiu Ing-Ming. Exchange of genetic sequences of long terminal repeat and the env gene by a promiscuous primate type D retrovirus. Virus res. 2003, vol. 96, no.1-2, pp. 107-111

6. Ikeda S. ; Tsutsui K. ; Hatsushika M. ; Watanabe S. ; Oda T. Purification and biochemical characterization of squirrel monkey retrovirus-H produced in a human lymphoblastoid cell line. 1992, vol. 38, no 5-6, 653-659

7. Middleton PG; Miller S; Ross JA; Steel CM; Guy K. Insertion of SMRV-H viral DNA at the c-myc gene locus of a BL cell line and presence in established cell lines. Int J Cancer. 1992 Sep 30;52(3):451-4