ESTABLISHMENT AND CHARACTERISTICS OF A CELL LINE FROM FETAL BOVINE THYROID (FBTY)

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Received September 3, 1993

Abstract

Fischer O., M. Machatková, L. Pecůchová, D. Zendulková and Z. Hořínová: *Establishment and Characteristics of a Cell Line from Fetal Bovine Thyroid (FBTY).* Acta vet. Brno, 63, 1994: 81–87.

Characteristics of a cell line from fetal bovine thyroid were tested in the 150th passage. The line was adapted to growth in the minimal essential medium supplemented with 10 % bovine fetal serum and it had an epithelioid morphology. After seeding of $1x10^4$ cells/ml, the culture density was higher than $1x10^6$ cells/ml on day 6. The FBTY line was heteroploid. The average number of chromosomes was 48.8 ± 10.0 and only 1 % of mitoses had a diploid number of chromosomes (2n = 60). Species specificity (Bos taurus) was confirmed by indirect immunofluorescence. Besides pronounced fluorescence with rabbit antisera against bovine proteins, weak reactions with antisera against ovine, caprine and swine proteins were observed. The FBTY line was susceptible to two strains of parainfluenza 3 virus, six strains of infectious bovine rhinotracheitis virus and bovine adenoviruses of the serovars 1, 2, 3, and 8.

Mitosis, indirect immunofluorescence, bovine adenovirus

Thyroidal cell cultures are suitable for the replication of adenoviruses. Benkö et al. (1989) described the cell line ECTC derived from fetal bovine thyroid gland which was, in passage 150, susceptible to all known serovars of bovine adenoviruses, including members of the subgroup II which are difficult to replicate.

In 1981, Marie Machatková in the Veterinary Research Institute, Brno, established a cell line from fetal bovine thyroid gland and the results are described in this paper.

The characteristics of this cell line (FBTY) were tested in passage 150.

Replications of bovine adenoviruses in FBTY and some other bovine cell cultures were compared by Pecůchová (1993).

Materials and Methods

Establishment and culture

Thyroid gland of a nine-month-old male bovine fetus was collected under sterile conditions immediately after exsanguination (Machatková and Pospíšil 1975) and transported to the laboratory in minimal essential medium (MEM SEVAC, Institute of Sera and Vaccines, Praha) supplemented with 200 I. U. of penicillin and 200 μg of streptomycin per ml. Tissue fragments were washed in the medium and cultured in 60 mm plastic Petri dishes in 5 ml of the EPL SEVAC medium (Institute of Sera and Vaccines, Praha) containing 10 % bovine fetal serum, 100 I. U. of penicillin and 100 μg of streptomycin per ml. The cells were cultured at 37 °C in an atmosphere containing 5 % CO_2. The EPL was replaced after 72h by a 1:1 mixture of EPL+MEM subsequently changed every other day.

As soon as the attached fragments were surrounded by growing cells, the cells were released by 0.02 % versene with 0.25 % trypsin Difco (1:250), reseeded into Mueller's flasks and cultured further in MEM with 10 % bovine fetal serum, 100 I. U. of penicillin and 100 μg of streptomycin per ml in 300 ml and 500 ml Roux flasks, Legroux flasks and Mueller's flasks.

In passage 85, the cells were frozen and kept for 8 years (1981–1989) in liquid nitrogen. When reseeded after passage 85, the cells were released using a solution of 0.02 % versene and 0.12 % chymotrypsin.

Elimination of mycoplasmas

Penicillin and streptomycin were replaced by ciprofloxacin (Bayer, Leverkusen, Germany) in a dose of 10 μg per ml of medium during 5 passages (113th through 117th) and the cells were cultured in an antibiotic-free medium during 30 subsequent passages (118th through 147th) (Fischer et al. 1989, 1992 a.). Besides the culture methods (Jurmanová and Machatková, 1986), the fluorescence methods based on staining of nucleic acids by bisbenzimide 33258 (Hoechst) (Machatková et al. 1986) or a derivative of acridine orange (AMHA) (Zendulková et al. 1992; Fischer and Zendulková 1993) were used for the detection of mycoplasmas in the cell

cultures during this period. Certain measures were taken in the course of cultivation to prevent any new contamination by mycoplasmas (Fischer 1992).

Evaluation of growth characteristics

The FBTY cells in passage 150 were seeded at a density of $1x10^4$ per ml into 60 mm plastic Petri dishes containing 4 ml of medium and incubated at 37 °C in an atmosphere containing 5 % CO_2 for 7 days. The cells were released and counted in 3 randomly selected dishes daily. The results of four independent tests were averaged.

Cytogenetic examination

The cells were seeded into Mueller's flasks with 5 ml of medium at a density $1x10^6$ cells per ml. After 6 hours of cultivation at 37 °C, 0.1 ml of a 0.01 % solution of colchicine was added to the medium. After another 10 h, the cells were released and prepared for examination by the method of Srb (1975).

At least 100 mitoses were examined in passages 100 and 150, chromosomes were counted and the average number of chromosomes, the modal number of chromosomes and the number of the cells with the diploid chromosome number were determined.

Verification of species of origin

The species of origin of the line FBTY was confirmed by a modified indirect immunofluorescence test described by Stulberg et al. (1961). Antisera were prepared from the blood of rabbits immunized with bovine, ovine, caprine or porcine spleen cells (Fischer et al. 1989).

Besides FBTY cells, cell cultures listed in Table 2 were also tested. Cell suspensions prepared from monolayers were washed by centrifugation in PBS, pH 6.5, at 1 000 rpm, incubated in test tubes with 0.2 ml of rabbit antiserum at 37 °C for 1 h, washed twice in PBS and incubated for 1 h with 0.2 ml of SwAR Ig FITC SEVAC conjugate (Institute of Sera and Vaccines, Praha) diluted 1:20. After washing with PBS, the cells were mounted into glycerol buffer, pH 5.5 (Machatková et al. 1986) and examined with a fluorescence microscope at a magnification of 252 x.

As controls the cells were incubated with the serum of non-immunized rabbits + SwAR Ig FITC SEVAC conjugate or with the conjugate alone.

The yellow-green fluorescence on the cell surface (Stulberg et al. 1961; Shiow et al. 1974; Fridlyanskaya et al. 1988) was classified as pronounced (+) or weak (±). Non-fluorescent cells (–) appeared as gray ground shadows hardly discernible from the dark background.

Experimental infection with selected bovine virus strains

Six strains of infectious bovine rhinotracheitis, two strains of parainfluenza 3 and eight strains of bovine adenovirus obtained from the Collection of Animal Pathogenic Microorganisms (CAPM) of the Veterinary Research Institute, Brno, were used for experimental infection of the FBTY cell cultures (Table 3).

Methods of experimental infections were described elsewhere (Pecůchová 1993). Briefly, monolayers of FBTY cells were washed with PBS. For the inoculation of approximately 48 cm^2 of the monolayer in Legroux flask, 0.5 ml of a virus suspension was used and after 1 h adsorption at 37 °C, the suspension was poured off and 20 ml of serum-free MEM was added to the monolayer. The flasks were incubated at 37 °C and checked daily for the cytopathic effect using light microscopy. Virus replication was also examined by the electron microscope Tesla BS 500 using negative staining with 2 % ammonium molybdate.

Results

Establishment and culture

Tissue fragments readily attached to the bottoms of the dishes and after 5–7 days of culture were surrounded by growing cells. The cells adapted well to MEM, were released readily and could be further cultured. After passage 60, epithelioid cells (Fig. 1) replaced spindle-shaped cells predominant in the initial passages.

Growth characteristics

The cells were viable after freezing and 8-year storage in liquid nitrogen. A high proliferation potential was evident at the passage 150.

After seeding $1x10^4$ cells ml, the density grew 34-fold, 68-fold and 116-fold on days 4,5 and 6 of culture, respectively.

At reseeding, the cells released easily from the surface of the glass vessels so that 0.25 % trypsin could be replaced by 0.12 % chymotrypsin.

Mycoplasma elimination

Ciprofloxacin completely eliminated the contamination of cells with unidentified mycoplasma species. No mycoplasmas were detectable after 2 passages in the ciprofloxacin-supplemented medium, during 30 passages in an antibiotic-free medium and in passages 150, 156 and 159.

No bacteria or fungi were found in the FBTY cell cultures.

Cytogenetic characteristics

The FBTY is a heteroploid cell line with a very low proportion of cells with diploid chromosome number (Table 1). The average number of chromosomes in passage 100 was 50.1 ±4.6, the range was 40–67 and the modal number of chromosomes was 53.

Cells with a diploid chromosome number constituted 2 % of the population.

The average number of chromosomes in passage 150 was 48.8 ± 10.0, the range was 33–96 and the modal number of chromosomes was 48. Only 1 % of mitoses had a diploid number of chromosomes (2n = 60).

Male chromosomes Y were apparent among the chromosomes during mitoses (Fig. 2).

Table 1

Cytogenetic characteristics of the cell line FBTY

Passage	100	150
Number of examined mitoses	100	200
Range of chromosomes	40–67	33–96
Average number of chromosomes	50.1±4.6	48.8±10.0
Modal number of chromosomes	53 (in 12 % of mitoses)	48 (in 14.5 % of mitoses)
Cells with diploid chromosome number (2n = 60)	2 %	1 %

Verification of species of origin

Intensive yellow-green fluorescence (+) of the surface was observed in FBTY cells incubated with the rabbit anti-bovine serum (Fig. 3), while incubation with anti-caprine (Fig. 4), anti-ovine or anti-porcine sera resulted in a weaker fluorescence (±). Bovine cell lines AU-BEK, FTL, MaBu and MDBK reacted in the same way as FBTY (Table 2).

Table 2

Verification of species of origin of FBTY cells by indirect immunofluorescence

Name and characteristics of cell culture		Reactions with antisera			
		bovine	ovine	caprine	porcine
FBTY	fetal bovine thyroid	+	±	±	±
AU–BEK	bovine kidney	+	±	±	±
FTL	bovine kidney	+	±	±	±
MaBu	bovine cornea	+	±	±	±
MDBK	bovine kidney	+	±	±	±
OVIR	ovine iris	±	+	±	±
CH-TM	caprine thymus	±	±	+	±
PK (15)	porcine kidney	±	±	±	+
CHO-K 1	Chinese hamster ovary	–	–	–	–
MA–104	rhesus monkey kindey	–	–	–	–
RK–13	rabbit kidney	–	–	–	–

No reactions of the cells with the serum of non-immunized rabbit or with the conjugate SwAR Ig FITC SEVAC alone were observed.

Ovine iris cells (OVIR) (Fischer et al., 1992b) reacted strongly (+) with anti-ovine serum and weakly (±) with anti-bovine, anti-caprine or anti-porcine sera.

Caprine thymic cells (CH-TM) reacted strongly with anti-caprine serum and weakly with anti-bovine, anti-ovine or anti-porcine sera.

Porcine kidney cells (PK [15]) reacted strongly with anti-porcine serum and weakly with anti-bovine, anti-ovine or anti-caprine sera.

Chinese hamster (CHO-K1), rhesus monkey (MA-104) and rabbit (RK-13) cells did not react (–) with any of the antisera (Table 2). None of the examined cell lines reacted with the serum of non-immunized rabbits + conjugate or with conjugate alone.

Comparisons of the fluorescence of the cell line FBTY and other cell cultures confirmed that the line FBTY originated from the species *Bos taurus* (*Artiodactyla: Bovidae*).

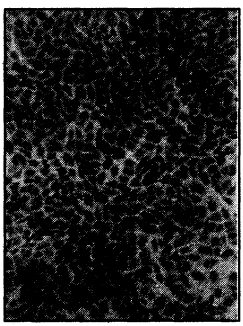

Fig. 1 FBTY cells, Giemsa, x 120.

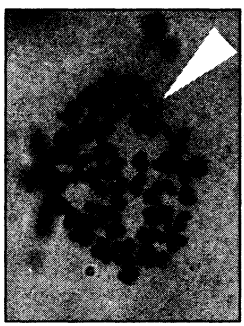

Fig. 2 Male chromosome Y is apparent among the chromosomes of dividing FBTY cell (arrow). x 1 000.

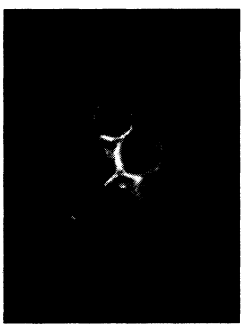

Fig. 3 Intensive fluorescence (+) of FBTY cells after incubation with bovine antiserum and conjugate. x 252.

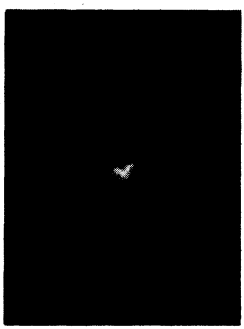

Fig. 4 Weak fluorescence reaction (±) of FBTY cells after incubation with caprine antiserum and conjugate. x 252.

Susceptibility to viruses

The cell line FBTY was susceptible to all the six strains of infectious bovine rhinotracheitis virus and both strains of parainfluenza 3 virus (Table 3). Virus replication was reflected in the development of cytopathic effect, and the presence of virus particles in the cells was demonstrated by electron microscopy. Of the eight adenoviruses, only the strains B-10, B-19, and WBRI (all are members of subgroup I) and Misk/67 (member of subgroup II), but not the strains Nagano, THT/62, 671130, and Fukuroi (members of subgroup II), replicated in the cell line FBTY (Table 3).

Table 3

Susceptibility of FBTY cells to experimental infections of selected bovine virus strains

Virus	Serovar	Subgroup	Strain	Cytopathic effect	Detection by electron microscopy
Bovine	1	I	B–10	+	+
adenovirus	2	I	B–19	+	+
	3	I	WBRI	+	+
	4	II	Nagano	–	–
	4	II	THT/62	–	–
	6	II	671130	–	–
	7	II	Fukuroi	–	–
	8	II	Misk/67	+	+
Infectious					
bovine			CAPM V–425	+	+
rhinotracheitis			CAPM V–426	+	+
			CAPM V–427	+	+
			CAPM V–428	+	+
			CAPM V–429	+	+
			CAPM V–430	+	+
Parainfluenza 3			CAPM V–30	+	+
			CAPM V–92	+	+

Discussion

Unlike the cell line ECTC derived from a cell suspension prepared by trypsinization (Benkö et al. 1989), the line FBTY was derived from cells proliferating from attached tissue fragments. Even the most careful trypsinization procedures are associated with a massive death of cells and the primary cell population rises usually from 0.1 to 10 % of the cells present in the initial tissue (Michl 1977). The more gentle derivation from tissue fragments was chosen in our experiments to avoid an exposure of the tissue to excessive selection pressure.

Spindle-shaped cell were predominant in bovine cell cultures after passage 5 in experiments performed by Jurmanová et al. (1975). Morphological transformation of the cell line FBTY, i. e., replacement of spindle-shaped cells by epithelioid cells, did not occur until passage 60.

FBTY cells are readily releasable and have a high proliferating potential, which are properties important for their practical use.

Infection of donor animals may be the source of mycoplasma contamination of cell cultures (Schwöbel and Leach 1970; Pfützner and Otto 1992).

Benkö et al. (1989) used the antibiotic BM cyclin for the elimination of mycoplasmas from the cell line ECTC. In our experiments, ciprofloxacin, which proved to be effective against Mycoplasma orale and M. arginini (Fischer et al. 1989, 1992a), was used to obtain the mycoplasma-free cell line FBTY.

Changes of karyotype of cultured bovine somatic cells were investigated by Sarkar et al. (1966). The cells with diploid chromosome number (2n = 60) predominated still in passage 19, constituting 76.4 % of the population. The cell line FBTY was heteroploid in passages 100 and 150, cells with diploid chromosome number constituted only 2 % and 1 %, respectively. As reported by Benkö et al. (1989), diploid number of chromosomes was found only in one of 86 mitoses in passage 150 of the cell line ECTC.

Verification of species of origin of cell lines by indirect immunofluorescence test is one

of the methods used in the Animal Cell Culture Collection in the U.S.A. (Stulberg et al. 1970). Cross reactions between antisera and cells of related animal species, e. g., monkeys and men belonging to the order Primates, were reported by Brand and Syverton (1960).

Our verification tests of the line FBTY demonstrated a specific reaction with anti-bovine and cross reactions with antiovine, anti-caprine, and anti-porcine sera. Chinese hamster, rhesus monkey and rabbit cells did not react with any of the four antisera. Cattle, sheep and goat (family *Bovidae*) and swine (family *Suidae*) are classed with the order *Artiodactyla*, while hamster (*Cricetulus griseus*) with *Rodentia*, rhesus monkey (*Macaca mulatta*) with *Primates* and rabbit (*Oryctolagus cuniculus*) with *Lagomorpha*. Therefore their cells did not react with the antisera to proteins of even-hoofed animals (*Artiodactyla*).

While the line ECTC is susceptible to all known serovars of bovine adenovirus, only 3 strains of subgroup I and 1 strain of subgroup II replicated in the line FBTY. Our results have confirmed the fact that cell lines from the same type of tissue may differ in their properties.

Pecůchová (1993) compared sensitivities of FBTY, bovine fetal testicular primary cell cultures, bovine fetal testicular cell line IB/T, bovine fetal turbinate cell line BTU/C (Fischer et al. 1990) and cell strain MaBu, which originated from bovine adult cornea (Fischer et al. 1992b), to bovine adenovirus strains. Bovine fetal testicular primary cell cultures were sensitive to all 8 strains (Table 3), the cell line IB/T to the strains B-19, 671130 and Fukuroi, the cell line BTU/C to the the strains B-10, B-19, WBRI, 671130, Fukuroi and Misk/67, the strain MaBu to the strains B-10, B-19, WBRI, Nagano, 671130, Fukuroi and Misk/67. Bovine fetal testicular primary cell cultures and the cell cultures BTU/C and MaBu were more sensitive to bovine adenovirus strains than FBTY.

Thyroid cells can proliferate in clusters if the medium is supplemented with hormones, especially TSH (Fayet et al. 1970; Szabó et al. 1991). No hormones were added into the medium during the isolation of FBTY and the cells grew only in monolayers.

Acknowledgements

The authors thank Mr. Lubomír Valíček for electron microscopy examinations. Skilled technical assistance of Mrs. Zora Němčanská, Mrs. Helena Mušková, Mrs. Jindřiška Mrvová, Mrs. Zdena Mikulášková and Mr. Jiří Kudrna is highly appreciated.

Založení a charakteristika buněčné linie štítné žlázy bovinního plodu (FBTY)

Byly testovány vlastnosti buněčné linie štítné žlázy bovinního plodu ve 150. pasáži. Linie je adaptována k růstu v mediu MEM s 10 % fetálního telecího séra a má epiteloidní morfologii. Po nasazení $1x10^4$ buněk/ml dosáhla 6. dne kultivace hustoty větší než $1x10^6$ buněk/ml. Linie FBTY je heteroploidní. Průměrný počet chromozomů byl 48,8±10,0 a pouze 1 % mitóz mělo diploidní počet chromozomů (2n = 60).

Příslušnost k druhu Bos taurus byla potvrzena nepřímou imunofluorescencí s použitím králičích antisér. Kromě výrazné fluorescence s antiséry proti bílkovinám skotu byly pozorovány slabé reakce s antiséry proti bílkovinám ovce, kozy a prasete. Linie FBTY byla vnímavá ke dvěma kmenům viru parainfluenzy 3, šesti kmenům viru infekční bovinní rhinotracheitidy a k bovinním adenovirům serovarů 1, 2, 3 a 8.

Основание и характеристика клеточной линии щитовидной железы плода крупного рогатого скота (FBTY)

Проверяли свойства клеточной линии щитовидной железы плода крупного рогатого скота на 150 пассаже. Линия приспособлена росту в среде MEM с 10% плодной телячьей сыворотки и отличается эпителоидной морфологией. После ввода $1x10^4$ клеток/мл на 6 сутки культивирование достигло плотности больше $1x10^6$ клеток/мл. Линия FBTY являертся гетеро-

плоидной. Среднее число хромосом достигало 48,8 ± 10,0 и лишь 1 % митозов отличалось диплоидным количеством хромосом (2n = 60).

Принадлежность к виду Bos taurus была подтверждена косвенной иммунофлюоресценцией с применением антисывороток кролика. Помимо выразительной флюоресценции с антисыворотками против белков скота наблюдали небольшие реакции с антисыворотками против белков овцы, козы и свиньи. Линия FBTY отличалась восприимчивостью по отношению к двум штаммам вируса парагрипозного заболевания 3, шести штаммам вируса инфекционного ринотрахеита скота и аденовирусам скота сероваров 1, 2, 3 и 8.

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