Isolation and Cultivation of Canine Transmissible Sarcoma Cells

Yoshiharu OKAMOTO, Toru FUJINAGA, Motoshi TAJIMA, Nobuhiko HOSHI, Kanjuro OTOMO and Toshio KOIKE

Department of Veterinary Surgery and Veterinary Anatomy, Faculty of Veterinary Medicine, Hokkaido University, Kita-18, Nishi-9, Kita-ku, Sapporo 060, Japan

(Received 12 February 1987/Accepted 12 September 1987)

ABSTRACT. The cells obtained from canine transmissible sarcoma (CTS) gradually divided into two types of floating and adherent cells after cultivation. From their histological findings and karyotype analysis, the floating cells were confirmed as CTS cells and the adherent cells were identified as fibroblasts. However, the floating cells were difficult to maintain in vitro beyond 2 weeks under the present cultivation system. CTS developed in all three dogs at the sites implanted of the floating cells, but no neoplasms appeared with the adherent cells.—KEY WORDS: canine transmissible sarcoma, dog.

Canine transmissible venereal sarcoma is a neoplasm on an external genitalia of both sexes transmitted by sexual contact. The disease is also transplantable homologously by subcutaneous inoculation with the tumor cells [3, 7, 8, 15, 18]. Successful transfers of this tumor in nude and X-ray-irradiated mice were reported [12, 17, 21]. Purification of the tumor cells and establishment of its cell line in vitro are required for further investigations of biological properties of the tumor.

This paper describes an attempt to cultivate the tumor cells and their transmissibility.

MATERIALS AND METHODS

Tumor: Canine transmissible sarcoma (CTS) was used in the present study. The CTS, which originated in a 7-year-old female dog, Hokkaido-inu suffered from canine transmissible venereal sarcoma in Sapporo, Japan [15], has been maintained for more than 90th passages by subinoculation in dogs since 1967.

Cell culture: Single cells from CTS were obtained by the same method described previously [15]. The cells were washed 3 times with phosphate buffered saline (pH 7.2) by centrifugation at 150×g for 5 min. Viability of them was determined by the trypan blue exclusion test. After centrifugation, they were resuspended in tissue culture medium to give 5×10^5 cells/ml. The culture medium consisted of RPMI 1640 (Gibco Lab., New York), serum, 2×10^{-5} M 2-mercaptoethanol, 1 mM oxalacetic acid, 0.2 I. U. insulin/ml, and 5 mg streptomycin/ml. Three ml of the cell suspension were dispensed into Petri dishes (Corning, New York) and the cultures were incubated at 37°C in humidified air with 5% CO_2.

Light microscopy: The culture cells were placed on a slide glass by a cytocentrifuge. After air drying, they were fixed in ethanol, stained with Giemsa solution for 20 min at room temperature, and examined morphologically.

Electron microscopy: The culture cells were fixed in 2.5% glutaraldehyde, and collected by centrifugation. The resulting cell pellets were covered with a drop of 0.5% soft agar. They were then cooled,
fixed in 1% OsO₄, and embedded in Quetol-812. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with HITACHI HU-12A electron microscope at accelerating voltage of 75 kV.

**Histological examination:** The tissues of the neoplasms were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were then prepared and stained with hematoxylin and eosin by routine procedure.

**Chromosomal examination:** Chromosome of the culture cells were analysed by the same method described previously [14].

**Inoculation of the culture cells:** The cells derived from CTS were cultured in vitro for 7 or 30 days and were inoculated subcutaneously on the abdominal right side of three dogs (6 months, female). The fresh cells obtained from CTS were also inoculated similarly on the left side of the dogs as control.

**RESULTS**

**Cell culture:** The cells derived from CTS gradually divided into two types of floating and adherent cells in the culture vessel after incubation. The floating cells were round or oval in shape and their sizes were range of 10 to 12 μm in diameter. Their nuclei contained one or two prominent nucleoli. On electron-microscopic examination, the surface of floating cells showed irregularity with microvillous projections. The cytoplasm was abundant, while the nucleus was characterized by a large nucleolus (Fig. 1). The chromosomal number of them was 58 as a modal number.

On the other hand, the adherent cells
were fibroblast-like cells. The nuclei of them were round or oval in shape. No prominent nucleolus was observed in their nuclei. Their chromosomal number was 78 as a modal number. The adherent cells were subjected successfully to at least 20 passages with subculture in every 10 days.

Conditions of cell culture: Volume and frequency of exchange of culture medium, containing fetal calf serum (FCS), calf serum (CS) or horse serum (HS) were examined. The culture media were exchanged as follows: 1/4 volume of medium in every 4 days, entire volume of it in every 4 days, 1/4 volume of it in every 2 days, 1/2 volume of it in every 2 days, and entire volume of it in every 2 days.

The viable cell number and viability of floating cells were shown to decrease gradually in culture medium containing FCS or CS in all of the exchanging conditions. However, no change was observed in both of the indices with culture medium containing HS in any of the exchanging conditions. From these results, it was decided that 1/4 volume of culture medium was exchanged in every 4 days for the following examinations.

The viable cell number and viability of floating cells after incubation are shown in Fig. 2. The former decreased gradually by 14 days after incubation using culture medium containing HS, but the latter did not decrease thoroughly for at least 2 weeks. Both of the values did not differ among culture media containing 10 to 40% HS and were slightly improved at a medium of pH7.2 than at pH7.4, and at an incubation temperature of 37°C rather than at 39°C using culture medium containing 20% HS.

Mitotic frequency of the floating cells: The mitotic frequency of floating cells is shown in Fig. 3. The average of it was 6.8 cells per 1,000 cells from the 1st to the 7th day after incubation and was 3.7 cells from the 8th to the 14th day.

Inoculation of the culture cells: The floating and adherent cells which were obtained after cultivation of cells derived from CTS used for experimental inoculations. The floating cells were cultured for a week, while the adherent cells were cultured for 30 days at the 3rd passages in every 10 days. Both groups of the cells (2×10⁶), an equal mixture of each cell group (1×10⁶) and the fresh CTS cells (2×10⁶) were prepared as inocula.

Neoplasms of 2~3 mm in diameter appeared at the sites inoculated with the
fresh CTS cells, the floating cells, and the equal mixture of floating and adherent cells in the three dogs on one week after inoculation. After that their growth patterns were almost the same among the three inocula. However, no neoplasms grew at the injection sites of the adherent cells by 4 weeks after inoculation.

Neoplasms caused by the fresh CTS cells and other two inocula were indistinguishable histologically (Fig. 4). CTS cells which were characterized by a large and round or oval nucleus with one or two prominent nucleoli were observed microscopically in the neoplasms. Lymphocytes and a few fibroblasts were scattered among CTS cells. Mitotic figures of CTS cells were frequently observed.

DISCUSSION

Experimental studies on CTS are at present based on materials obtained from in vivo. These materials, however, consist of many different cells including tumor cells. The use of CTS cells with other kinds of cells presents a problem in investigation areas of this condition. Although tissue cultures have been tried by many workers [1, 4, 16, 19, 20], propagation of CTS cells in vitro had been unsuccessful in most cases, and the tumor cells were replaced by normal fibroblasts contaminated in culture cells.

In this study, the culture cells derived from CTS were divided into two types, floating and adherent cells. The results of morphological and karyotype analyses revealed that most of the floating cells showed the major characteristics of CTS cells reported [1, 5, 6, 10, 11, 13, 14, 15]. On the other hand, the adherent cells were identified as fibroblasts derived from connective tissue in the tumor because of their growth pattern, morphological characteristics and chromosomal number. No other cells except CTS cells were observed among the floating cells on the 3rd day after incubation.

Mitosis of the CTS cells was noticed in vitro without an accompanying increase in cell number until 2 weeks after incubation. One possible explanation is that the number of propagating cells is fewer than it of dying cells. It was reported that floating cells derived from various tissues could not be maintained for a long term in primary culture, except in body fluids in vivo [9]. Although studies on the culture of CTS cells have been attempted by various methods such as using several kinds of cells as a feeder layer, adding other chemical factors into the culture medium, and others, the results of the present study showed that the floating CTS cells may be difficult to maintain in vitro beyond 2 weeks, even if the cells were incubated under the suitable conditions showed in the present study.

Adams et al. [1] succeeded in propagating CTS cells in vitro for a long term. However, they failed to transfer CTS to dogs using these culture cells [1, 2]. The present study indicated that the floating cell produced CTS, which is significant because there was no reports on the inoculation of CTS cells obtained from cell culture. The adherent cells, on the other hand, disappeared at the injection sites.

REFERENCES


要約

犬の可移植性肉腫（CTS）細胞の分離と培養：岡本芳晴・藤永 徹・田島晋士・星 信彦11・大友勲十郎・小池壽男（北海道大学獣医学部家畜外科学講座，11家畜解剖学講座）——CTS 由来細胞は培養開始後短期間浮遊細胞と付着細胞に分れ、形態学的特徴と染色体数から、浮遊細胞が CTS 細胞であり、付着細胞は線維芽細胞と考えられた。培養開始 3 日後の浮遊細胞から CTS 細胞をほぼ純粋に得ることができ、培養条件を検討した結果、pH7.2 の 20％馬血清培養液を用いて 37℃で培養し、4 日毎に培養液を 1/4 交換する方法が適当と考えられた。しかし、この条件によって培養しても、2 週間を越えての浮遊細胞の維持は困難であった。浮遊細胞の犬への接種により CTS の発症が確認され、付着細胞による発症是認められなかった。