Implantation of Canine Transmissible Sarcoma Cells to X-Ray Irradiated and Nude Mice

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Canine transmissible venereal sarcoma cells were reported to be transplanted into X-ray irradiated and nude mice [5, 8, 11]. The neoplasms which grew at the inoculation sites were examined histologically, but their growth pattern and the karyotype of their cells were not distinguished.

In this paper, X-ray irradiated and nude mice were experimentally inoculated with the sarcoma cells and then the cells derived from the neoplasms which grew in a nude mouse were experimentally inoculated into dogs. The neoplasms which developed after these inoculations were examined histologically and the karyotypes of their cells were analysed.

Canine transmissible sarcoma (CTS) was used in the present study. The tumor, which originated in a 7-year-old female dog Hokkaido-inu with canine transmissible venereal sarcoma in Sapporo, Japan [7], has been passaged more than 90 times by serial subcutaneous implantation in dogs since 1967.

The animals used in this study were 72 ddY (3 weeks of age) and 5 nude (nu/nu, congenic strain of BALB/c, 3 weeks) mice. The ddY mice were exposed to general X-ray irradiation of 600 rads at a dose rate of 68.95 rad/min at 180kVp and 25 mA using an X-ray unit (Toshiba KXC-18, Tokyo). They were bred in an isolator after irradiation.

Single CTS cells as a inoculant were collected by the method described previously [7]. The viable cells were suspended in a concentration of $1 \times 10^7$–$5 \times 10^7$/ml with Ringer’s solution containing 2,000 I.U. of penicillin/ml and 20 mg of streptomycin/ml. Five hundred $\mu$l of the cell suspension were inoculated subcutaneously into the mice immediately after X-ray irradiation.

Stubbs, E. L. et al. [11] reported the transplanted CTS cells in mice which were exposed to 400 rads of general X-ray irradiation but not the age of the mice or the growth pattern of the tumor. In our preliminary experiments, the neoplasms which grew after inoculation with the CTS cells did not appear in 3 BALB/c mice (52 weeks) which were irradiated generally with 500 rads of X-ray, and each 2 mice (8 weeks) of the same strain that received 800 or 1,000 rads died. On the other hand, the CTS cells were transmissible to each 7 mice of 6 and 3 weeks of age of the same strain exposed to 600 rads. Therefore, the dose level of 600 rads was decided as standard. Effect of X-ray irradiation levels on the development of the neoplasms could not be discussed because the age and strain of the experimental mice could not be fixed in the preliminary and present experiments.

After inoculation of the CTS cells into the irradiated mice, 6 mice were sacrificed every 5 days for gross and microscopical examinations. The neoplasms were excised surgically and fixed in 10% neutral buffered formaline and embedded with paraffin. Sections were then prepared by the routine procedure and stained with hematoxylin and eosin. The chromosomal number was calculated by the method described previously [6].

Neoplasms were detected at the inoculation sites on the 5th day. They developed to the maximum size of 8.0 mm in average diameter by the 10th day after inoculation (ai). Their size gradually decreased to 2.5 mm in average diameter on the 30th day and 1.2 mm on the 60th day ai.

The neoplasms were grossly white in color and rigid and could be isolated from the surrounding tissues. Their central region, which was excised on the 5th and 10th day ai, was necrotic histologically. Infiltration of neutrophils was seen in the necrotic area, of which the marginal region contained mainly large and circular cells, with one or two prominent nuclei in their nucleoli. After the 10th day the large and circular cells had rapidly disappeared in the neoplasms and the

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necrotic tissue tended to be replaced with connective tissue.

The chromosomal number of the large and circular cells on the 5th day \( ai \) was 58 as a modal number (range of 55–61).

These morphological and karyotype findings showed that the large and circular cells had the major characteristics of CTS cells reported previously [1, 2, 3, 5, 6, 9, 10].

When the nude mice were inoculated with the CTS cells, the neoplasms grew very slowly, their diameter reaching approximately 6–8 mm on the 17th week \( ai \) and their size remaining almost unchanged until the 24th week \( ai \), the end of the observation period. On the 120th day \( ai \), the neoplasms constituted mainly large and circular cells by microscopic examination and their morphological features were the same as those of the CTS cells. In the neoplasms, lymphocytes and macrophages infiltrated moderately among the tumor cells, but no necrotic tissue was observed.

The neoplasms which grew in the nude mice were excised surgically on the 24th week \( ai \) and treated with 0.25% trypsin in phosphate buffered saline, pH 7.2. The cells were then suspended in RPMI 1640 (Gibco Lab., New York) supplemented with 20% horse serum, \( 2 \times 10^{-5} \) M of 2-mercaptoethanol, 1 mM of oxalacetic acid, 0.2 I.U. of insulin/ml, 200 I.U. of penicillin/ml and 5 mg of streptomycin/ml and incubated in sterile plastic Petri dishes at 37°C in humidified 5% CO₂ in air.

The cells obtained from the neoplasms divided gradually into two types of floating and adherent cells in the culture vessel after cultivation. The results of karyotype analysis of the floating and adherent cells were as follows: the chromosomal number of the former was 58 as a modal number (range 34–64), which was the individual number of canine transmissible venereal sarcoma cells. On the other hand, the chromosomal number of the latter was 40 as a modal number (range of 29–40), which was the same as that of one of the body cells of a mouse.

The floating cells on the 3rd day after incubation were inoculated subcutaneously into 3 mongrel puppies (4–6 months old). Neoplasms appeared at the injection sites of all the dogs by the 2nd week and grew to approximately 1 cm in average diameter in size by the 7th week \( ai \). Microscopically viewed, the central region of the neoplasm on the 80th day \( ai \) was occupied with necrotic tissue. Large and circular cells, which had a round or oval nucleus with one or two prominent nucleoli, were scattered in the margin of the necrotic area, and their cells were surrounded with lymphocytes and a few infiltrating fibroblasts. The microscopic findings of these large and circular cells could not be distinguishable from those of CTS cells. Chromosomal number of their cells was 58 as a modal number (range of 40–59).

The present results showed that the CTS cells were transmissible to X-ray irradiated and nude mice and that the CTS in the nude mouse could be returned to dogs. These findings were confirmed by histological and chromosomal examinations.

REFERENCES

要約

犬可移植性肉腫（CTS）のX線照射マウスおよびヌードマウスへの移植（短報）：岡本芳晴・藤永徹・田島聡士・大友勉十郎・小池壽男（北海道大学獣医学部家畜外科講座）—犬の可移植性肉腫由来 CTS 細胞を皮下接種された X線照射マウスでは、接種10日後直径約8mmの腫瘍が形成されて後、徐々に退縮した。ヌードマウスでは CTS の発育は緩慢で、接種17週後に最大直径約6〜8mmに達し、ヌードマウスから得られたCTS 細胞を幼犬皮下に継代接種したところ、接種7週後に直径約1cmの腫瘍が形成された。