146. **Further Karyological Evidence for Contageousness and Common Origin of Canine Venereal Tumors**

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(Comm. Oct. 12, 1974)

The canine venereal tumor of the external genitalia of both sexes has called special interest of oncologists and cytogeneticists on account of its unique nature: it is naturally transmissible from dog to dog through copulation. Since Watanabe et al. (1955), the chromosomes of this tumor have been extensively studied on both primary and transplanted tumors from several different localities in Japan as well as from abroad (see Makino 1963, 1974). It was shown that the stemline cells of different tumor samples exhibited an apparently uniform karyotype consisting of 59 chromosomes including 17 biarmed and 42 acrocentric elements, with minor numerical and/or structural variations, in striking contrast to the normal somatic complement of the dog which comprised of 76 acrocentric autosomes and 2 biarmed sex chromosomes. These pictures are consistent with the view that the canine venereal tumor is a naturally transplanted tumor which has arisen from one and the same origin, though the possibility that the karyological similarity is merely superficial has not completely been ruled out by conventional cytological techniques (Makino 1963).

Recently, we studied chromosomes of 2 primary and 1 transplanted canine venereal tumors obtained in Sapporo, by means of the quinacrine (Q) and Giemsa (G) banding techniques, with particular attention to the precise characterization of morphologically altered chromosomes in tumor cells (Oshimura et al. 1973), and the results

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*) Contributions from the Chromosome Research Unit, Hokkaido University. Supported by grants from the Ministry of Education, Japan.
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strongly supported the previous view. It is etiologically important to learn the similarity or dissimilarity of the chromosome banding pattern in tumor specimens derived from distantly separated locations. The present paper was prepared in response to this query.

**Table I. Chromosome number distribution in 7 primary canine venereal tumors**

| Case No. (Sex) | Locality    | Date of sampling | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 2s (%) | No. of cells observed |
|---------------|-------------|------------------|----|----|----|----|----|----|----|----|----|--------|----------------------|
| 4 (♀) Sapporo | Feb/13/73   |                  | 2  | 4  | 6  | 17 | 1  |    |    |    |    | 0 (0)  | 30                   |
| 5 (♂) Sapporo | Feb/22/73   |                  | 2  | 5  | 5  | 19 |    |    |    |    |    | 19 (38) | 50                   |
| 6 (♀) Nagasaki| May/11/73   |                  | 1  | 2  | 3  | 3  | 1  |    |    |    |    | 40 (80) | 50                   |
| 7 (♂) Nagasaki| Sept/73     |                  | 2  |    |    |    |    |    |    |    |    | 0 (0)   | 2                    |
| 8 (♂) Kagoshima| Oct/73     |                  |    |    |    |    |    |    |    |    |    | 0 (0)   | 1                    |
| 9 (♀) Sapporo | Nov/8/73    |                  | 2  | 4  | 22 | 11 | 4  |    |    |    |    | 7 (14)  | 50                   |
| 10 (♂) Nagasaki| Apr/74     |                  | 1  | 6  | 12 | 11 | 15 | 1  |    |    |    | 0 (0)   | 50                   |

2s=double stemline cells

**Materials and methods.** Seven primary tumors were collected from 3 different locations; 3 from Sapporo, 3 from Nagasaki and 1 from Kagoshima. They were obtained at different occasions during a 14 months period, from 3 male and 4 female dogs (Table I). PHA-stimulated blood cultures of a normal male dog were served as control.

Chromosome slides were prepared according to the routine air-drying method (Oshimura et al. 1973). The Q-staining (Caspersson et al. 1970), C-staining (Sumner 1972) and N-staining (Matsui and Sasaki 1973) methods were employed for banding chromosomes. The conventional Giemsa-stained slides were made for examination of the chromosome number and centromeric position.

**Results.** The chromosome numbers of the 7 tumors fluctuated between 54 and 62 with modal values at 57 (1 case), 58 (1 case) and 59 (5 cases), while a considerable number of polyploid cells with a duplicate set of the stemline complement was noted in 3 cases (Table I). The stemline cells of each tumor were characterized by having 15–18 biarmed chromosomes, among which the cells with 17 biarmed elements predominated. The biarmed chromosomes were morphologically similar in different samples, except that the third largest element was submetacentric in case 4, while it was nearly metacentric in the other cases. In all of the 7 cases, the long arm of the second largest submetacentric element was outstanding by negative heteropycnosis. The same arm showed a very faint fluorescence after Q-staining (Fig. 1), whereas it stood out deeply stained in C- and N-
stained samples (Fig. 2). All polyploid cells had 2 such large chromosomes with a distinctive C-band or N-band positive long arm (Fig. 3). In contrast, no comparable features were observed in normal somatic chromosomes of cultured blood cells (Fig. 4). Most interphase nuclei of all tumor samples showed a prominent chromatin body deeply stained after C- or N-banding. This body was usually observed closely associated with the nucleolus, and there were 2 such
bodies in presumed polyploid cells (Fig. 5).

Q-banding patterns of 5 largest biarmed and 2 largest acrocentric elements were compared in 5 cases (cases 4, 5, 6, 9 and 10), based on 2-4 informative plates in each case. As in previous cases (Oshimura et al. 1973), only a few tumor chromosomes retained apparently normal morphology with normal banding patterns: this suggests that extensive structural rearrangements had occurred in tumor chromosomes. Careful comparison of banding patterns in the 7 largest elements failed to detect any appreciable difference among the 5 tumor samples studied, except for the third biarmed element of case 4 (Fig. 1). This element was comparable in both morphology and banding pattern to the third largest submetacentric element in case 3 of our previous report (Oshimura et al. 1973).
Remarks. Our data indicate that canine venereal tumors show a striking karyotypic similarity even in samples from distantly separated locations like Nagasaki and Sapporo, and that the similarity is not superficial as suspected before (Makino 1963). Further evidence is thus provided for the hypothesis that this tumor is arisen from a common origin by cellular contagion (Watanabe et al. 1955, Makino 1963, 1974).

It seems that the stemline karyotype of this tumor is highly stable as shown by general similarities of banding patterns in several larger chromosomes from different samples, even though there are some minor variations. Similar extensive structural changes of chromosomes have been shown by banding analysis in some long-lasting transplantable animal tumors such as the Ehrlich and Yoshida ascites tumors (Sasaki et al. 1974). The cell population of the Ehrlich ascites tumor showed an extreme karyotypic heterogeneity, while that of the Yoshida sarcoma was relatively homogeneous and stable like the present canine tumors.

We have previously pointed that the large heteropycnotic submetacentric element is a common marker of canine venereal tumors which functions as a nucleolar organizer in interphase cells (Oshimura et al. 1973). The N-banding analyses of both metaphase and interphase cells strongly support the above view, since the N-banding method has been known to stain differentially the nucleolus organizing regions (Matsui and Sasaki 1973).

Summary. The karyotypic uniformity is demonstrated by Q-, C- and N-banding techniques in 7 primary canine venereal tumors obtained from 3 distantly separated locales in Japan, in support of the previous view that the canine venereal tumor is a naturally transplanted tumor derived from the same origin.

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