Chromosome Analysis with Special Reference to Centromeric Heterochromatin and Ploidy Variation in Mouse Sarcoma-180 cells

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Studies on the role of chromosome in the process of cancerogenesis is of continuous importance in the field of cancer cytogenetics. Sevral reports on chromosome analysis of solid tumours have been published of which contributions made by Makino (1957), Kato (1968), Atkin and Ross (1960), Levan (1969) and Mitelman (1971) are quite alluring. Chromosomal analysis of sarcoma cells has been reported mostly on rat and Chinese hamster (Yosida 1949, Makino 1957). Mouse sarcoma, on the other hand, has received as yet less importance, in the karyological literature of neoplastic cells. However, Levan (1954), Nombela and Murcia (1971), Nielsen (1976) and others have analysed the chromosomal changes in some ascites tumours of mouse. Sarcoma 180, originally developed spontaneously as carcinoma in the breast of a female mouse, was subsequently developed to sarcoma by transformation of the connective tissue cells (Woglom 1964). The present study has been oriented to analyse the chromosome complement of mouse Sarcoma 180 cells with special reference to centromeric heterochromatin and to the nature and extent of ploidy.

Materials and methods

The ascitic form of sarcoma 180 strain was transplanted to inbred Swiss mice with an inoculum size of $5 \times 10^7$ cells. The chromosomes were prepared after the 3rd day of transplantation by following the technique described elsewhere (Chakrabarti and Chakrabarti 1977a). In order to detect C-heterochromatin, C-banding was performed by slight modification of the technique originally suggested by Sumner (1972). One exception is that Barium-hydroxide-ocatahydrate (5%) treated slides were kept in $4 \times SSC$ (instead of $2 \times SSC$) for 45 minutes only at 60°C.

Observations

An analysis of 300 well-spread metaphases from 30 different specimens revealed that the chromosome complement of sarcoma 180 cells was highly variable. Quantitative analysis of metaphases showed that the majority of the cells were aneuploid and the chromosome count varied from hypohaploidy i. e., below 20 (Fig. 3).
polyploidy i.e., above 480 (Fig. 4). The frequency values of the stemline number indicated 75 (Figs. 1, 2) as the modal chromosome number which represented 14% (Fig. 5) of the total cell population. The marginal aneuploids varied between 70 to 76 which constituted about 39.5% of the total metaphases examined. The cells nearest to the modal number consisted of 76 and 74 chromosomes with the frequency 8% and 5.5% (Fig. 5), respectively. The frequency of cells with chromosome complements below stem line number (i.e., less than 70) was 35%, whereas the frequency of cells with the chromosome number higher than mode values (above 76) was 25% only.

The morphology of chromosomes of sarcoma 180 has been analysed only on the basis of reliable and well-spread metaphases of the stem cells. Conventional Giemsa staining revealed that the cell line consisted of one biarmed marker
Fig. 2. Karyotype of MS-180 from conventional Giemsa stained metaphase. a) marker submetacentric. b) and c) pair not identifiable.

chromosome (Figs. 1, 2) which has been formed by the Robertsonian fusion of two unequal acrocentric elements (Figs. 1, 2, 7a), and the remaining 74 were all acro-or telocentric elements (Figs. 1, 2) which also included a few minute chromosomes (Figs. 1, 2, 6). In many cells, the minutes were not very easily identifiable due to their dot-like appearance. The number of these minutes varied from 1 to 3 in
most cells (Fig. 6).

Detection of C-heterochromatin: ‘C’ banding analysis of mouse sarcoma 180 cells revealed a number of peculiarities which were not identifiable by conventional Giemsa staining. C banding revealed that a) the marker sub-metacentric was actually a dicentric chromosome with two closely situated C-positive heterochromatic zones (Fig. 7a). b) An apparently ring shaped chromosome whose pair was not identifiable in the Giemsa karyotype (Figs. 1 and 2) showed distinctly terminal C positive heterochromatic zones (Fig. 7b). The position and nature of C-heterochromatin in this chromosome indicated that the chromosome was actually a dicentric with two terminal centromeres. c) Another chromosome which showed

![Histogram indicating the frequency values of different chromosome counts of MS-180 cell line.](image)

an elongated appearance in conventional Giemsa preparations, revealed a characteristic extended C-heterochromatin (Fig. 7c). Although most of the chromosomes showed the existence of C-positive centromeric region yet some of them also lacked the same (Fig. 7).

Discussion

The present study is significant for many reasons. The modal number of mouse sarcoma 180 was previously determined as 88 with a range between 71 to 172 by Shannon and Macy (1972) from their study with the solid form of this tumour. They failed to find the presence of any marker chromosome in this particular cell
line. But the present study clearly detected that the ascitic form of MS 180 possesses a modal number of 75 with a distinct submetacentric marker chromosome (Figs. 1, 2, 7a). Critical C banding analysis revealed that this submetacentric was actually dicentric in nature with two closely situated centromeres (Fig. 7a), and had arisen by Robertsonian fusion between two nonhomologous chromosomes prob-

Fig. 7. C-banded metaphase from MS-180 showing (a) submetacentric marker (dicentric), (b) dicentric with terminal centromeres and (c) unusual chromosome with extended C-heterochromatin.

ably during the transformation of the tumour from solid state to ascitic condition. Moreover, in addition to this biarmed marker, there was one more dicentric chromosome with two terminal centromeres (Fig. 7b). Previously it has generally been argued that dicentric chromosomes are unstable (See White 1977). But in our investigation we have recorded the existence of these dicentric chromosomes in
all cells even after several successive *in vivo* passages of the tumour which is indicative of the fact that these unusual dicentrics may also be stable and can perpetuate normally along with other chromosomes of the cell. Like other tumours, the occurrence of extreme ploidal variation has also been recorded in the present study. Critical analysis has revealed that the occurrence of aneuploidy is mainly due to chromosome elimination during anaphase separation, and tandem fusion as exhibited in Figs. 8 and 9 respectively. However, like other tumours (Oksala and Therman 1974), the existence of multipolar spindle has also been noticed (Fig. 11) in this particular cell line.

The high frequency of polyploidy as exhibited in Fig. 4 is due to endoredupli-
cation (Fig. 10) which is very common feature in neoplastic cells (Levan and Hauschka 1953, Atkin 1976).

Summary

Chromosome analysis of an ascitic form of mouse sarcoma 180 has been made both by conventional Giemsa staining as well as by C-banding. The modal number has been found as 75 with one biarmed marker in the present ascitic tumour. C-banding analysis reveals that this submetacentric marker is actually a dicentric chromosome with two closely situated C-positive heterochromatic zones. In addition to this marker, the cell line also possesses another stable and transmissible dicentric chromosome with two distinctly terminal centromeres, and another unusual chromosome with an extended centromeric heterochromatin.

Spontaneous chromosome elimination during anaphase separation, endoreduplication, multipolar spindle formation, etc. have been recorded in a high frequency which are assumed to be responsible for the characteristic ploidal variation in this cell line.

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Literature cited

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