83. *Chromosome Studies in Mouse Neuroblastoma Cells*

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A mouse neuroblastoma C1300 (Stewart *et al.* 1959) having been in serial transplantation since 1940, was adapted to *in vitro* culture in 1967. A number of clonal lines have been established with regard to their neuronal properties (Augusti-Tocco and Sato 1969; Klebe and Ruddle 1969; Hamprecht 1977). Karyological analyses in some of those cell lines have been carried out with special attention toward the correlation between the cellular function and chromosomal constituents (Hamprecht 1977; Amano *et al.* 1972; Amano 1975; Minna *et al.* 1972). There is however only a report that mentioned about the occurrence of double minutes (DMs) (Warter *et al.* 1974). We have chanced to observe DMs variable in number and morphology, together with some other aberrant chromosomes in several C1300 cell lines. Reports on DMs, which usually appear as paired, and very small chromatic bodies, have increasingly been accumulating in recent literature in malignant cells (Olinici 1971; Sandberg *et al.* 1972; Biedler *et al.* 1973; Levan and Levan 1975; Levan, A. *et al.* 1977; Levan, G. *et al.* 1977; Balaban-Malenbaum and Gilbert 1977; Barker and Hsu 1979; Quinn *et al.* 1979). Series of cytogenetic and cytochemical studies in murine ascites tumors (Levan, G. *et al.* 1977; Levan, A. *et al.* 1978; Levan and Levan 1978), as well as in human carcinoma cell lines (Barker and Hsu 1978, 1979) have been made an outstanding contribution to understanding of the structure and behavior of DMs. However, on the origin and significance of DMs our knowledge has not been fully established. DMs have been recorded so far in the majority of human tumors, most frequently in neuroblastoma, but less in animal tumors. This report presents some additional evidence for the DMs observed in mouse neuroblastoma cells.

Materials and methods. The N18TG-2 and NS20Y cell clones used were obtained through the courtesy of Dr. M. Nirenberg at NHLBI, NIH, U.S.A., and the C1300 solid tumor and NB2A clone through Dr. T. Kato at the Nagoya City University School of Medicine, Nagoya. The C1300 tumor was easily converted into *in vitro*

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growth. All cells were grown as described (Nelson *et al.* 1969).

Conventional hypotonic methods were used for the most part of karyotype analyses: The cells were arrested by 0.01–0.02 μg/ml of Colcemid solution for 3 hrs at 37°C, swelled in 0.075 M KCl solution for 30 mins at room temperature, fixed in methanol-acetic acid (3:1), and air-dried on slides. G- and C-banding techniques followed Deaven and Peterson (1974).

Findings and remarks. The results of chromosome analyses in 4 cell lines studied are shown in Table I. The karyotype of C1300 tumor was first reported in 1956 to have 66–70 chromosomes (Ref. Hauschka *et al.*). Recently this tumor was reported to have 60 to 63 chromosomes (Warter *et al.* 1974) and 64 chromosomes at mode (Amano 1975). In the present passages, cells cultured from the solid tumor had a modal number of 63, ranging from 62 to 64, thus with a stability in the chromosome pattern during over 20 year-passages of growth. However, three clones derived from C1300, such as N18TG-2 (inactive clone), NS20Y (cholinergic clone) and NB2A (adrenergic clone), showed the modal values near tetraploidy, having 81, 97 and 72 chromosomes. Although their karyotypes were apparently different from one to others, they were coincident in the fact that all contained distinct marker elements, large-sized metacentric and/or submetacentric in outline, varying from 5 to 11 in modal number (Figs. 1, 4 and 5). As seen in Fig. 1, extensive chromosome rearrangements have occurred, together with a wide range of cell to cell karyotype variations. The chromosomes of the N18TG-2 line has been investigated by other authors (Minna *et al.* 1972; Hamprecht 1977), but the present communication is the first for the NS20Y and NB2A lines.

The most striking features found in metaphase of neuroblastoma

Table I. Karyological characteristics of mouse neuroblastoma C1300 cell lines

Cell line	No. of cells examined	Chromosome number mode (range)	Marker chrom. mode (range)	MCs*) range	DMs	
					Range	%**)
C1300	22	63 (62–64)	5 (4–6)	1–2	1–5	47
	3	(116,134,136)	(11–12)			
N18TG-2	96	81 (56–111)	7 (5–11)	1–7	1–15	89
	6	(169–182,351)	14 (11–17)		102	
NS20Y	23	97 (92–106)	6 (4–7)	1–3	1–2	21
	2	(189,197)	(8,12)	4–5		
NB2A	13	72 (70–91)	11 (9–15)	1–4	1	<10
	1	(145)	(19)			

*) Microchromosomes. **) Indicates average population of cells with DMs.

cells were the presence of DMs. The DMs appeared as small, and paired chromatin bodies which stained darkly with Giemsa (Fig. 2), lightly with G-banding (Fig. 5), while negatively stained with C-banding, in similar manner to many other tumors with DMs (Barker and Hsu 1979). The number of DMs per metaphase cell varied from 1 to 100 or more, and the average population of cells with DMs also varied by clone ranging from less than 10% (NB2A) to about 90% (N18TG-2) (Table I). The DMs usually scattered all over the metaphase plates. Sometimes, however, they were found attached to the ordinary chromosomes or forming clusters (Fig. 3), as reported pre-

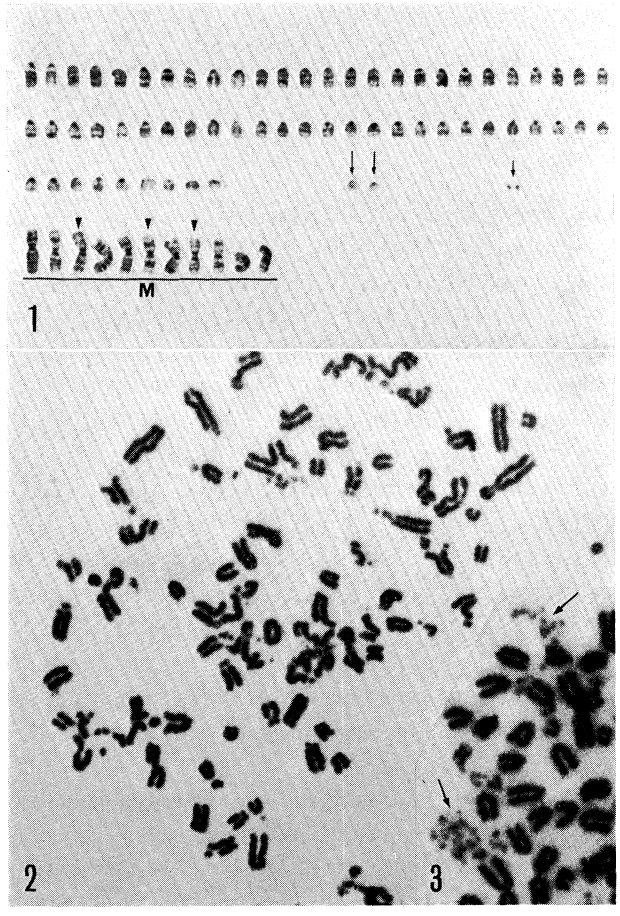
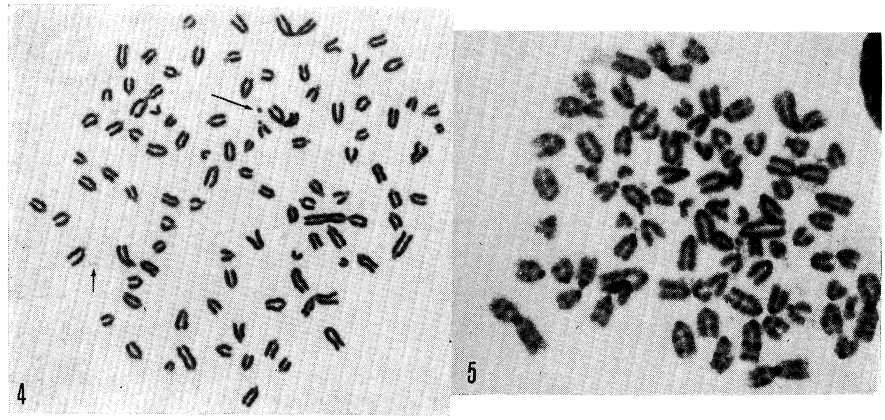


Fig. 1. Karyotype of an NB2A cell with 72 chromosomes, 2 micro-chromosomes (large arrows) and a DMs (small arrow). Eleven markers (M) with no HSR, three of them (arrowheads) consisting of fused isochromosomes are present.

Figs. 2-3. Metaphases from C1300. 2: Note many DMs of various sizes. 3: Showing clusters of DMs (arrows).



Figs. 4–5. 4: Metaphase from NS20Y with 94 chromosomes, a microchromosome (large arrow) and a faintly stained DMs (small arrow). Five markers are present. 5: G-banded metaphase of N18TG-2 with 81 chromosomes. DMs stain lightly.

viously (Mark 1967; Barker and Hsu 1978; Levan and Levan 1978).

In addition to the DMs, most of cells contained another type of minute chromosomes referred to as microchromosomes (Levan *et al.* 1977). They were single, C-band positive and relatively larger than DMs, but smaller than the ordinary chromosomes, and showed less numerical variety by cell (Table I). The co-existence of both types of minutes has been reported in certain tumors (Donner and Bubenik 1968), though their nature has also remained unknown. The cells here studied contained microchromosomes, 1 to 7 in number. Then it is evident that there is no direct correlation between the number of chromosomes and that of DMs. But there is a possible trend in showing that polyploid cells carried larger numbers of DMs more frequently than cells with less chromosomes or low levels of ploidy.

Another distinctive chromosome aberration was a pulverization of single or a few chromosomes observed in neuroblastoma cells. The pulverized chromosomes appeared as faintly stained, extended or highly fragmented element. In the extensive studies of DMs in Rous sarcomas, Mark (1967) has emphasized the possible mechanism of the DMs formation as a result of chromosome pulverization.

Recently, evidence has increasingly been presented suggesting a close relationship between DMs and homogeneously staining regions (HSR) in relation to their origin and function (Levan *et al.* 1977; Balaban-Malenbaum and Gilbert 1977; Quinn *et al.* 1979). HSR in the present materials were hardly recognizable. Most reasonable speculation from available evidence is that DMs or HSR may represent amplification of specific genes favorable for tumor growth

(Levan *et al.* 1977; Barker and Hsu 1979). If that is the case, specific gene or genes amplifying by the DMs in C1300 cells were unknown at the moment, though it was evident that DMs in the C1300 cells are of chromosomal origin.

Summary. Chromosome studies were carried out in cultured cells from a mouse neuroblastoma C1300 tissue and in three clones established from this tumor. They possessed characteristic karyotypes with remarkable markers. Double minutes (DMs) were demonstrated in all cell lines, in addition to some other chromosomes aberrations, such as microchromosomes and chromosome pulverization.

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