Homogeneously Staining Regions in Direct Preparations from Human Neuroblastomas

Gloria Balaban and Fred Gilbert

Departments of Pediatrics and Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

ABSTRACT

Previous descriptions of homogeneously staining regions in human neuroblastomas have been in karyotypes obtained from established cell lines. We now report homogeneously staining regions in direct preparations from two human neuroblastomas. In one of the cases, the homogeneously staining region identified in the primary tumor was also found in metastases to bone marrow and pleural fluid. The homogeneously staining region is, therefore, not an artifact of growth in vitro.

RESULTS

Neuroblastomas have been staged on the basis of the extent of involvement with tumor (8). Stage IV involves metastasis to distant, discontinuous sites.

NB 19. A 14-month-old white female was diagnosed (December 29, 1977) as Stage IV neuroblastoma with a positive bone marrow and multiple bone lesions. No metaphases were available for analysis in the preparations obtained directly from the initial bone marrow aspirate. Chemotherapy began on January 2, 1978, and a remission was induced. Laparotomy on June 28, 1978, gave no evidence of disease at the primary site (right adrenal). The patient relapsed on January 10, 1979, at which time a second bone marrow was obtained. Subsequent courses of chemotherapy produced only partial responses. The patient died on June 17, 1979.

From the second marrow, postchemotherapy, only non-banded preparations were obtained. The predominant population had a mode of 46 chromosomes with varying numbers of double minutes per cell. Two cells had no double minutes; instead, they contained long marker chromosomes. Good banding of the preparations was not achieved until the cultured bone marrow cells were passaged as a tumor through nu/nu mice. Metaphases from the cell line established after explanation of the tumor back into tissue culture contained 2 or 3 HSRs per cell. In each cell, one HSR was found on 13p, and the other HSRs were on marker chromosomes the origin of which could not be established with confidence. The size and centromere position of one of the HSR-containing chromosomes was the same as for one of the long marker chromosomes seen in the second, direct bone marrow preparation.

Fig. 1A is an unbanded metaphase from the second, direct marrow preparation with arrow to the long marker chromosome. Fig. 1B is an unbanded metaphase from the same preparation (the second, direct marrow) with arrow to the double minutes. Fig. 1C is a banded metaphase from the cell line derived from the tumor in a nu/nu mouse (second bone marrow cells injected into mouse with the resulting tumor explanted back into culture); arrows point to HSR-containing chromosomes.

NB 56. A 15-month-old white male was diagnosed as Stage IV neuroblastoma (August 1979) and begun on chemotherapy with initial improvement. Laparotomy performed several months later revealed an unresectable primary in the left hemiabdomen (originating from the left adrenal). The patient also had a mediastinal mass, pleural effusions, and metastases to the calvarium. Tissue samples were obtained (March 1980, after therapy had begun) from the bone marrow and pleural effusion for chromosome analysis. Multiple courses of chemotherapy were unable to induce a remission and the patient died April 18, 1980. At autopsy, a tissue sample was obtained from the left adrenal primary.
The karyotypes obtained from all 3 sources (primary tumor and metastases to bone marrow and pleura) were similar. Two cell populations were evident in the preparations: the predominant near-diploid population; and a second, tetraploid population. Individual cells were missing or contained extra single chromosomes; all cells contained a HSR in a compound chromosome, with the HSR extending between 13q and 9q (confirmed by C- and Q-banding).

Fig. 2 shows a banded karyotype from bone marrow aspirate with arrow to the HSR-containing chromosome. Fig. 3A shows the metaphase from primary tumor with arrow to the HSR; Fig. 3B is the C-banded metaphase which confirms that the HSR-containing chromosome does include 9q heterochromatic material, as well as 13q heterochromatin; Fig. 3C is the hypotetraploid metaphase from pleural effusion with arrows to HSRs (2 copies of the 13/9 HSR).

DISCUSSION

The HSR was first identified in 2 human neuroblastoma lines in 1976 (4). In one of the lines, IMR-32, the same long marker chromosomes could be seen in the original unbanded chromosome preparations made soon after the line was established in culture in 1967 (Ref. 20 and Footnote 4; the HSR was clearly evident in banded preparations from a recent reconstitution of the earliest freeze of the line). It was, therefore, possible that the HSR in this case arose in the tumor itself.

Subsequent reports of HSRs in neuroblastomas have been from permanent cell lines (1, 7, 19). To establish whether HSRs can occur in the neuroblastoma in vivo, we began an analysis of karyotypes obtained directly from the tumors and from metastases. We have now identified HSRs in direct preparations from 2 independent tumors. Although both of these patients had received chemotherapy prior to removal of the tissue samples for study, an earlier report from this laboratory described HSRs in 2 neuroblastoma cell lines (IMR-32 and CHP-126) established from untreated patients (1). The HSR, therefore, does occur in the absence of exposure to antimetabolites or other chemotherapeutic agents.

The HSR was first reported in direct preparations from mammary, esophageal, and pharyngeal neoplasms by Kovacs (11). We have now demonstrated HSRs in direct preparations from human neuroblastomas and, for the first time, have identified the specific HSR-containing chromosome in a complete, banded karyotype from a direct preparation.

In one of the cases described in this paper, NB 56, the same HSR was identified in preparations from bone marrow and pleural effusion as well as primary tumor. Because the cells from all 3 sources shared the same marker rearrangements, they were all derived from a common cell. We can, therefore, conclude that the appearance of a HSR may precede metastatic spread of the tumor.

In previous reports of HSRs in karyotypes from human neuroblastomas, the cells from which permanent lines were established were derived primarily from bone marrows or from other metastases (1, 4, 7, 19). This also raises the possibility that the presence of the HSR may contribute to the capacity of a tumor to metastasize.

The functional significance of HSRs in human neuroblastomas is unknown. It has been proposed that the development of a HSR represents a mechanism for the amplification of particular genes (4). This hypothesis was proven to be correct in methotrexate-resistant cell lines, for example, in which it was shown that the HSRs contained multiple copies of the gene for dihydrofolate reductase (15).

However, the neuroblastoma cell lines with HSRs are methotrexate sensitive, implying that dihydrofolate reductase has not been amplified in these lines. That genes other than dihydrofolate reductase can be amplified in an HSR is indicated by the appearance of a HSR in a vincristine-resistant cell line and by the finding of multiple copies of the genes for RNAs in the HSR of another line (5, 13).

It is also significant that the HSRs in individual neuroblastoma cell lines have been stably associated with different chromosomes over the months to years that the lines have been in culture [with HSRs longer than any single band in the karyotype on chromosomes 1, 5, 6, 7, and 13 (1, 2, 4) and with shorter HSRs on other chromosomes (6)]. This raises the possibility that the HSRs on different chromosomes result from the amplification of different genes.

Studies to define the individual genes which have been amplified within HSRs, to determine how an HSR is produced (whether by unequal crossing over or by saltatory replication, as outlined in Ref. 17), and to establish the role(s) played by the HSR in cancer discussed in Refs. 9 and 10 are in progress.

REFERENCES

14. Nielsen, K. Chromosomal evolution in the Ehrlich-Lettre complex of hyper-
Fig. 1. A, NB 19. Unbanded metaphase from second bone marrow aspirate with arrow to long marker chromosome. B, NB 19. Metaphase from second bone marrow with arrow to double minutes. No HSR-containing chromosomes evident. C, NB 19. Metaphase from cell line described from tumor in athymic mouse (see "Results") with arrows to HSR-containing chromosomes.
Fig. 2. NB 56. Complete banded karyotype from bone marrow with arrow to HSR on 13/9 compound chromosome.
Fig. 3. A, NB 56. Metaphase from primary tumor with arrow to long marker chromosome. B, NB 56. C-banded metaphase from bone marrow with arrow to compound HSR-marker chromosome. C, NB 56. Metaphase (hypotetraploid) from pleural effusion with arrows to 2 HSR-containing chromosomes.
Homogeneously Staining Regions in Direct Preparations from Human Neuroblastomas

Gloria Balaban and Fred Gilbert


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/42/5/1838

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.